THE PERSISTENCE OF BACTERIAL AND VIRAL CONTAMINANTS ON THE SURFACE OF SEEDED PRODUCE AND THEIR REMOVAL USING MECHANICAL AND CHEMICAL TREATMENTS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2000

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By

Michael Lee Bradley

DEDICATION

This dissertation is dedicated to my wife, Dr. Jennifer Bradley, whose support, guidance and encouragement was never-ending, and to my son, Eli, who is the joy of my life.

I also wish to thank my parents, Harry and Lynn Bradley for their constant love and support, and for helping me keep things in perspective.

ACKNOWLEDGEMENTS

Funding by USDA Grant 98-34382-6927 *Mechanical and Antimicrobial Treatments to Remove Pathogens from Produce*

I wish to thank my co-workers in the Environmental Microbiology lab for their assistance. Special thanks to George Lukasik, Wei-Yea Hsu, Salina Parveen, Hannah Kaufman, Heleen Wittusen, and Diane Andryshack.

I would like to acknowledge my committee members Dr. Doug Archer, Dr. Robert Bates, Dr. Samuel Farrah, and Dr. Rachel Shireman for their support.

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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December 2000

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Outbreaks of foodborne illness associated with consumption of fresh or minimally processed produce items are becoming an all too frequent occurrence. The attachment of these pathogens to the surface of produce items, their ability to persist under a variety of conditions, as well as their responses to disinfection treatments have not been adequately explored to date. Four produce items with distinctly different surface characteristics were selected for examination: strawberries, tomatoes, romaine lettuce, and cantaloupe. The pathogenic bacteria *Escherichia coli* O157:H7 and *Salmonella* Montevideo, both implicated in outbreaks associated with produce, along with the bacteriophages MS2, PRD1, Phi-X 174, and the enterovirus polio 1 were selected as challenge organisms. The ability of the bacteria and viruses to persist on tomatoes and strawberries as influenced by temperature, location, degree of processing (i.e., waxing), and organic load was examined. Efficacy of disinfection treatments, including chlorine and chlorine dioxide solutions, was evaluated. Challenge

organisms were recovered up to 21 days post-inoculation from tomatoes, after 9 days on strawberries, and challenge organisms on both fruits were resistant to disinfection treatments evaluated. Romaine lettuce was inoculated with challenge organisms to identify localization preferences, examine their ability to persist under refrigerated storage, and evaluate efficacy of disinfection treatments. Inocula demonstrated a propensity toward attachment to leaf tip and midrib portions of pre-cut lettuce and persisted throughout the 14-day study. Statistically significant reductions were obtained by all the disinfection treatments examined, including chlorine solutions applied over a broad range of pH. Localization of challenge organisms on inoculated cantaloupe was examined with preferential attachment observed for the stem scar. Disinfection treatments were examined and greater population reductions on cantaloupe surfaces were observed than for the other products examined. Bacteria and viruses were recovered up to 30 days post-inoculation from cantaloupe. The results of this study indicate bacteria and viral agents readily attach to produce surfaces and survived for extended periods under normal storage conditions. While significant reductions were obtained by the treatments evaluated, complete elimination of challenge organisms was not achieved. These findings stress the necessity to prevent produce contamination rather than relying on disinfection procedures.

CHAPTER I
INTRODUCTION

The exchange of produce among international trading partners has provided consumers with a plethora of choices in the produce aisle; however, it has also created concerns about the safety of those products. These produce items include intact fresh fruits and vegetables as well as minimally processed items which may have been washed, sliced, and packaged by the producer, packing house or retail grocer. The dietary practices of Americans have changed over the years as well. Public health information campaigns such as the food pyramid and the "Five-a-Day for Better Health" as promoted by the National Cancer Institute, have succeeded in increasing the consumption of fresh fruits and vegetables by the American public (Hedberg et al., 1994). In their review of national reports of foodborne illness and the factors associated with those illnesses, Hedberg et al. examined changes that could be contributing to the increase in illnesses associated with the consumption of raw and minimally processed fruits and vegetables. These factors include changing consumption patterns, and the use of wastewater and inadequately composted manure on soils used for fruit and vegetable production. Other factors, such as the inadvertent introduction of pathogens into new geographic locations due to increasing globalization of the food supply, and even the development of new virulence factors by microorganisms has been implicated as contributing to the increase in foodborne illness associated with produce (Beuchat 2000)

Produce may become contaminated in a number of ways It may be contaminated while growing in fields or orchards, during harvesting or post-harvest handling or by infected food handlers or poor food handling practices (Beuchat, 1996 2000) The risk to consumers posed by contamination of the exterior surface of produce is two fold First, pathogens are ingested directly such as with ready to eat (RTE) produce where the exterior is not peeled away. In this case the product serves as a fomite i e vector that does not support pathogen growth This mechanism may occur with almost any fresh or minimally processed fruit or vegetable. The key to this pathway is that the pathogen is able to survive the inhospitable conditions experienced during storage and transport, and is able to resist washing and/or sanitization processes

In the second instance exterior contamination can be transferred to the interior of the product, during slicing or processing, as demonstrated by previous studies of pathogenic bacteria (Ukuku and Sapers 2000, Suslow et al , 2000, Lin and Wei 1997) Once the bacterial or viral pathogens gain access to the interior of the product, pH and other factors are antagonistic toward most pathogens but do not ensure product safety (Parish 1997) This is the pathway that is more likely to be of concern in a food processing setting, such as the slicing of melons or tomatoes either in a home, retail food establishment, or commercial produce operation.

Bacterial and viral infections associated with the consumption of fruits and vegetables are a growing concern for consumers and public health organizations (Cliver, 1997, CAST, 1994). Pathogenic bacteria such as *Salmonella spp.* and *Escherichia coli* O157:H7 have been linked to the consumption of produce including watermelons, cantaloupes, sprouts, tomatoes, and fresh salad vegetables (Altekruse et al., 1997, Bean et al., 1996, Council for Agriculture Science and Technology [CAST], 1994). Viral pathogens, such as human enteric viruses, have also been implicated in a variety of produce-borne diseases and are among the leading causes of foodborne illness (Altekruse et al., 1997, Bean et al., 1996, Dahling et al., 1993, Jaykus, 1997, Mead et al., 1999). Infected individuals can excrete these viral pathogens in high numbers, and although viruses will not grow on or in food products, they can persist on foods.

There are several gaps in knowledge associated with produce safety that need to be addressed. The first is elution techniques and methods to recover pathogenic bacteria and viruses from various produce items. Techniques that will elute the maximum number of the target organisms (either inoculated challenge organisms, or naturally occurring contaminants) allow for more representative surveys of produce in the market, as well as provide a sound basis for experimentation with produce in the laboratory. Techniques in the recovery and quantification of microorganisms from water and some surfaces have been developed and widely used. However, differences in surface structures and the internal composition of produce items pose obstacles in the processing of samples for analysis and create the need to develop specific techniques to address these challenges. In this study, elution techniques and recovery systems reported in the scientific literature were applied to produce items with varying composition.

There has been much work in the field of horticulture concerning the localization and attachment of various plant pathogens (Bartz and Showalter 1981). This work has not been done to a great extent, however, with microorganisms of concern to human health. Plant pathogen studies have also included survival studies of plant pathogens or spoilage organisms artificially inoculated onto various produce items. But again, the survival of bacteria and viruses associated with foodborne illness has not been studied in detail, especially incorporating environmental variables such as temperature and organic load influences such as the nutrients present in waste water. The present study evaluated the persistence of pathogenic bacteria and selected viruses over a variety of storage variables including temperature, inoculum carrier and location of inoculum application, and degree of processing applied to the produce type.

The recent incidence of illness associated with the consumption of produce has made consumers more aware of the potential risks posed by fresh produce items prepared and consumed at home. Limited scientific information is currently available about the effectiveness of treatment methods available to consumers to reduce microbiological contamination. This study examined methods that can be used at the home or retail levels, as well as commercially, to reduce the amount of bacterial and viral contamination on produce items.

CHAPTER II
LITERATURE REVIEW

There has recently been an increase in the research being conducted in the field of produce safety due to the increasing incidence of produce-implicated illnesses. This chapter will address a few of the topics in the current scientific literature that are relevant to this study, with information presented in four parts: a review of the incidence of produce-borne illness and the produce type implicated, a review of relevant research methodology regarding produce safety, and a brief overview of disinfection treatments available to address produce contamination and a discussion about the localization of and survival of bacteria and viruses on the surfaces of produce.

Illness associated with produce consumption. The increasing incidence of illness associated with the consumption of produce has been well documented (Altekruse et al., 1997; Bean et al., 1996; Cliver, 1997). Beuchat (1996, 2000) has provided two thorough overviews of both reported foodborne illnesses associated with produce, and a review of the current research associated with produce safety. Strawberries have been associated with repeated outbreaks of hepatitis A during the 1990s. An outbreak of hepatitis A linked to the consumption of frozen strawberries in Georgia and Montana during a four-month period in 1990 was traced back to a single processing plant (Niu et al., 1992). In 1997, frozen strawberries distributed through the USDA sponsored school lunch programs were implicated in a total of 153 cases of hepatitis A in Michigan (Centers for Disease Control and Prevention [CDC], 1997). The strawberries implicated in the Michigan outbreak were first received from Mexico, and were then processed, packaged, and distributed by a single company in Southern California (CDC, 1997).

Tomatoes have been implicated in several outbreaks of foodborne illness as well. Outbreaks of Salmonellosis associated with tomatoes occurred in 1991 and 1993 (Hedberg et al., 1994; Wood et al., 1991). Tomatoes have also been associated with viral infections, with diced tomatoes being implicated in an outbreak of hepatitis A (Williams et al., 1994).

Outbreaks of *E. coli O157:H7* infections have been linked to the consumption of lettuce. A 1995 outbreak of *E. coli O157:H7* in Western Montana was traced back to the sources of the implicated lettuce, and poor agricultural practices, such as the use of improperly composted dairy manure and unprotected irrigation waters were identified (Ackers et al., 1998). A second well-documented multi-state outbreak of *E. coli O157:H7* associated with mesclun lettuce occurred in 1996. The contaminated lettuce was traced back to a single source, and field investigators identified numerous poor agricultural and manufacturing practices potentially contributing to the contamination of the product (Hilborn et al., 1999).

Cantaloupes imported from Mexico were implicated in the 1997 outbreak of *Salmonella saphra* infections (Mohle-Boetani et al., 1999). An outbreak of *Salmonella chester* in 1990 was also traced back to the source of the cantaloupes from Mexico (Ries et al., 1990). Domestically produced cantaloupes have been associated with foodborne illness as well, with cantaloupes from Texas growers implicated in an outbreak of *Salmonella poona* (CDC, 1991).

These outbreaks of foodborne illness highlight the potential for bacterial and viral pathogens associated with produce to enter the domestic food supply, be widely distributed, and cause illness to those consuming the contaminated products. The increased consumption of fresh produce and subsequent increasing incidence of foodborne illness are creating concerns for consumers and food safety officials alike about the microbial quality and safety of produce (Altekruse et al., 1997; CAST, 1994; Tauxe et al., 1997). It should be kept in mind that while the number of confirmed cases of illness attributed to produce is increasing, these incidents are still relatively infrequent compared to the number of cases associated with foods of animal origin. Nonetheless, findings from this review of the literature have demonstrated the importance and need for additional research related to produce safety and preventative measures.

Current research methods associated with produce safety. The selection of challenge organisms to incorporate into disinfection and survival studies can be a source of great debate among members of the scientific community. While there are standard methods for the evaluation of disinfectants, along with specified reference organisms (AOAC, 1995), these may not be readily applicable to the evaluation of produce disinfection treatments. Researchers working with a combination of challenge organisms often use a selective medium containing supplements to differentiate individual bacterial species (Lukasik et al., 2000; Castillo et al., 1999; Finn and Upton, 1997; Jacxsens et al., 1999). Differentiation is also accomplished using antibiotic-resistant strains, such as rifampicin-resistant strains of *E. coli* and *Salmonella* (Lukasik et al., 2000; Castillo et al., 1999; Lin and Wei 1997; Wei et al., 1995).

While selective and differential media may be utilized to distinguish the challenge organisms from the background flora of the produce, the first challenge researchers face is the removal of the challenge organisms from the inoculated produce. Lukasik et al. (2000) provided an excellent overview and comparison of both elution techniques and elution and recovery media to elute *Salmonella*, *E. coli*, and viruses from tomatoes and strawberries. These methods for the elution of challenge organisms (bacteria and viruses) from the surfaces of strawberries and tomatoes as well as elution and recovery media were incorporated into this study.

The recovery media for the challenge bacteria consisted of trypticase soy agar supplemented with rifampicin, ferric ammonium citrate, and sodium thiosulfate. The antibiotic rifampicin reduces the background microflora of the samples, while the sodium thiosulfate and ferric ammonium citrate allow the bacterial colonies to be distinguished from each other (Lukasik et al., 2000). The eluent buffer reported by these researchers, phosphate-buffered saline supplemented with the neutral detergent Tween 80, was also utilized in this study. The salt and detergent combination was reported to surmount the electrostatic and hydrophobic forces that facilitate the attachment of the challenge organisms to the surfaces of produce. The method of Lukasik et al. (2000) was also applied to cantaloupe, which was inoculated and processed in a manner somewhat similar to tomatoes.

The elution method of Lukasik et al. (2000) for the recovery of challenge organisms from strawberries and tomatoes (i.e., excising inoculated tissue) could not be readily applied to lettuce because of the physical differences in the structure of the products. Escudero et al. (1999) reported a method of inoculation and elution from chopped iceberg lettuce. The recovery method of Escudero et al. (1999) (stomaching) was compared to a modified version of the shaking method reported by Lukasik et al. (2000) for the elution of challenge bacteria and viruses from the surfaces of fresh-cut romaine lettuce. While the method of Escudero et al. (1999) eluted high numbers of challenge organisms the potential for the aerosolization of the challenge viruses associated with the lettuce due to foam production during stomaching was significant enough that the method of Lukasik (2000) was selected.

Viral pathogens, such as human enteric viruses, have also been implicated in a variety of produce-borne diseases and are among the leading causes of foodborne illness (Altekruse et al., 1997; Bean et al., 1996; Dahling et al., 1993; Jaykus, 1997; Mead et al., 1999). Undoubtedly, much more is known about bacterial pathogens versus viruses, due to deficiencies in viral isolation and identification techniques. For example, there are more than 100 types of human pathogenic viruses that can be present in water contaminated by human and/or animal fecal matter, but only a small portion can be readily detected by current methods (Bosh et al., 1988; Havelaar et al., 1993). Many viruses can be recovered from raw sewage effluent and treated sewage discharge (Cliver, 1997), and even soils and natural bodies of water (Bosh et al., 1988). However, in comparison to bacterial pathogens, there is less information about the physical association and removal of viral agents from produce surfaces (Beuchat, 1996; Jaykus, 1997).

In addition to the two representative bacterial pathogens *S.* Montevideo G4639 and *E. coli O157:H7* (ATCC 110195-B1), the bacteriophages Phi-X-174, PRD-1, and MS2 were included as challenge viruses in this study. Poliovirus 1 (strain Lsc) was also included in selected persistence and disinfection trials. Because of the difficulties associated with the recovery, isolation, and identification of many of the enteric viruses the use of selected viral strains and certain bacteriophages as indicators of viral contamination and as models for other pathogenic viruses have been used in research studies. The viruses included in this study have previously served as models of human enteric viruses in attachment and persistence studies (Lukasik 1998 Lukasik et al. 1998 Lukasik et al 2000 Davies et al 1993 Ferrah 1982 Funderburg and Sorber 1985 Havelaar et al , 1993 Jones et al , 1991) and disinfection trials (Davies et al 1993 Jones et al , 1991)

Disinfection treatments The protection of produce items from contamination presently includes the application of food protection systems such as Hazard Analysis and Critical Control Point Systems (HACCP) Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) In addition to these established food safety systems the U S Food and Drug Administration's Center for Food Safety and Applied Nutrition has produced the "Guide to minimize microbial food safety hazards for fresh fruits and vegetables" which is intended to serve as a guide for the production and processing of fresh produce (1998) This document is only a guide and is not currently compulsory The prevention of contamination of produce is a very difficult task and current food protection systems may not be enough to assure the production and distribution of pathogen free fruits and vegetables This may be especially true considering the volume of produce items imported from countries where GAPs and other food safety systems are not in place and under regulatory oversight.

While prevention of contamination is the preferred method of assuring produce is safe for consumption, disinfection treatments may also play an important role.

Washing fruits and vegetables with potable water immediately post-harvest as a part of a rapid cooling program at packinghouses prior to distribution, or immediately before consumption at the point of use is undoubtedly the most frequent treatment applied to produce Beuchat (2000) indicates that washing produce items in hot water removes a portion of the pathogenic and spoilage organisms present but that scientific studies evaluating the efficacy of this simple and prevalent treatment are few The study presented in this report included treatments of produce items with warm (43°C) tap water

While the evaluation of chlorine treatments applied to tomatoes artificially contaminated with *Salmonella* Spp (Wei et al , 1995, Zhuang and Beuchat, 1996) and other pathogens (Beuchat and Brackett, 1991) have been published in the scientific literature disinfection of produce surface associated viruses with chlorine solutions have not been reported Chlorine solutions are frequently used to disinfect produce items in commercial operations at concentrations of 50-200ppm with exposure times typically 1 2 minutes (Beuchat 2000) The effectiveness of 50, 100 200, and 300ppm free chlorine solutions at inactivating challenge bacteria and viruses on the surfaces of strawberries tomatoes, lettuce, and cantaloupe was evaluated The effectiveness of chlorine treatments is influenced by the amount of free available chlorine (as hypochlorous acid and hypochlorite ion), which is greatly influenced by pH (White, 1992). To examine this influence on the efficacy of the treatments, chlorine solutions were applied to inoculated lettuce with the pH unadjusted or adjusted to pH 7.3 for each chlorine concentration used.

The scientific information currently available on the effectiveness of chlorine dioxide is limited in comparison to the information available about the use of chlorine as a disinfectant for fruit and vegetables. Chlorine dioxide has been used to disinfect *Listeria monocytogenes* inoculated onto lettuce by Zhang and Farber (1996) and chlorine dioxide has also been evaluated for controlling microorganisms in water used in hydrocoolers for chilling cucumbers (Reina et al., 1995). To date there are no scientific studies published that explore the effects of stabilized chlorine dioxide products on viruses associated with produce surfaces. Chlorine dioxide was applied to inoculated strawberries, tomatoes, lettuce and cantaloupe in this study and the responses of the challenge bacteria and viruses examined.

This study examined methods currently utilized by some produce processors and packinghouses that can also be implemented at the home or retail levels to reduce the amount of bacterial and viral contamination from produce items. Carnebon 200® (International Dioxide Inc., Clark NJ), a commercially available stabilized chlorine dioxide product approved for use on produce items by commercial processors, was also examined.

Localization and persistence of challenge organisms. There has been much work in the field of horticulture concerning the localization and attachment of various plant pathogens. This work has not been done to a great extent, however, with microorganisms of concern to human health. The locations where transient microorganisms attach to lettuce, particularly fresh-cut lettuce, have only recently been explored, in part due to development of new microscopy techniques. Researchers using a combination of fluorescein isothiocyanate-labeled marker organisms and powerful microscopy techniques have documented not only the attachment to the surface of the leaf, but also the internalization of bacteria deep within the interior tissue (Seo and Frank 1999; Wood 2000; Takeuchi and Frank 2000). Studies have recently been conducted utilizing these methods to determine if the introduced flora demonstrate a propensity for attachment to differing regions of processed lettuce (i.e., cut surface or leaf tip) but have only looked at iceberg varieties of lettuce. This study examined the attachment preference of the challenge organisms on fresh-cut romaine hearts, midrib and leaf tips.

The attachment preferences of the challenge organisms to various locations on cantaloupes immersed in a suspension of challenge organisms were also examined. Intact surfaces from the top and bottom of the melons, and the stem and blossom scar regions were examined. Suslow et al. (2000) demonstrated that the intact rind of the cantaloupe is not an absolute barrier to artificially inoculated or indigenous bacteria, with *S.* Typhimurium LT2 recovered 5mm below the rind. Suslow et al. (2000) also reported that bacteria inoculated on the blossom and stem scars were occasionally detected in the interior flesh after storage. Determining the preferential attachment locations of bacteria and viruses may be a key point in the development of disinfection treatments or other preventative measures to address melon contamination.

There has also been considerable research done in the field of horticulture concerning the persistence of plant pathogens on plant surfaces. This work with plant pathogens has often included survival studies of plant pathogens artificially inoculated onto various produce items. But again, the survival of bacteria and viruses associated with foodborne illness has not been studied in detail, especially inclusive of environmental parameters such as temperature and organic load influences. While the survival of some human viral pathogens under a variety of conditions has been well documented (Abad, et al., 1994, Ansari, et. al., 1988, Dahling et al., 1993, Davies et al., 1993, Jones et al., 1991, Keswick et al., 1983), and the transmission of viral agents via fomites has been explored (Abad et al., 1994, Ansari et. al., 1988, Keswick et al., 1983), information pertaining to the association of produce and viral agents is scant (Beuchat, 2000, Jaykus, 1997). The persistence abilities and adherence preferences of relevant pathogenic bacteria and viruses on the surfaces of produce have not been studied in adequate detail; this is especially true for viruses. This study examines the persistence of two bacterial pathogens and selected viruses

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inoculated on strawberries, tomatoes fresh-cut lettuce and cantaloupes Variables including inoculum carrier (water or sewage) location of inoculation degree of processing and storage temperatures were applied to the produce items and their influences on challenge organism persistence examined

CHAPTER III
MATERIALS AND METHODS

Bacterial strains media and growth conditions Bacteria were stored at 70°C in 36% tryptic soy broth (TSB)(Difco Laboratories Detroit MI) The following strains of *E coli O157 H7* and the source of isolates examined were as follows American Type Culture Collection (ATCC) 43888 – isolated from human feces ATCC 35150 – isolated from human feces Kaspar 1404- derived from ATCC 43895– isolated from ground beef FRIK-24- isolated from cattle feces ATCC 110195 B1 – isolated from cattle feces. The following *Salmonella* Spp and their sources included *S* Montevideo G4639 – tomatoes, *S. Chester* ATCC 1197 – cantaloupe and *S Stanley* ATCC 7308 alfalfa sprouts Rifampicin-resistant strains of these bacteria were maintained on trypticase soy agar (Difco) containing 0 1 g/L of rifampicin (TSA rif) (Sigma Chemical Corp St Louis MO) The procedures for producing these rifampicin-resistant strains have been previously described (Wei et al 1995) For growth curve evaluation, one fresh colony (24hr) colony was inoculated into 5ml of TSB The tube was placed on a rotary shaker (Red Rotor Hoefer Scientific San Francisco, CA) with the cap slightly open and incubated at 36°C for 18 hours This resulted in a suspension of approximately 10^8 cells/ml One hundred (100) microliters of the suspension were inoculated into test tubes containing 5ml of TSB The tubes were then subjected to shaking at 30 rpm with the cap slightly open in a 36°C incubator

Each strain was grown in triplicate, and plated in duplicate at each time point. For all disinfection experiments, bacteria were prepared from static overnight cultures grown at 36°C in TSB. For persistence studies, the overnight suspensions were washed once in sterile saline (0.85%) to remove any remaining nutrients and cell debris and standardized to 10^7 colony forming unit per ml (CFU /ml) in sterile deionized water.

Bacteriophage and polio virus. Bacteriophage and their respective hosts were as follows: MS2 (ATCC 15597-B1) and *Escherichia coli* C-3000 (ATCC 15597), Phi X-174 (ATCC 13706-B1) and *Escherichia coli* (ATCC 13607), and PRD-1 and *Salmonella typhimurium* (ATCC 19585). Poliovirus was proliferated and assayed as plaque forming units (PFU) on Buffalo Green Monkey Kidney (BGM) cells.

Preparation of inoculation medium. Raw sewage was obtained from the University of Florida Water Treatment Facility, Gainesville, Florida. Sewage was prefiltered with a 5.0-µm porosity filter (Millipore GS, Millipore Corp., Bedford, MA) and then filtered with a 0.2-µm porosity filter (Millipore GS) which was pre-washed with 20ml of de-ionized water before use. Viral and bacterial cultures were diluted in sterile deionized water or sewage water to produce an initial titer of at least 10^7 CFU or PFU per milliliter. For persistence studies, bacterial cells which had been washed and suspended as previously described, were utilized. The temperature of the inoculation solution was maintained at least 15°C higher than the internal temperatures of all the produce items inoculated to reduce the potential internalization of the solution (Bartz and Showalter, 1981).

Strawberry preparation and inoculation. Strawberries were obtained from a local grocery store in Gainesville, Florida on the day of the experiment (Appendix 1). Berries weighing between 25-30g with no visible signs of surface trauma or tissue damage were selected and the leaves and stems removed. The strawberries were batch inoculated by immersion in 2L of deionized water (or filtered sewage) containing approximately 10^7 PFU/ml or CFU/ml of the bacterial or viral species for two minutes, with constant agitation. The fruit was then removed from the inoculum, and allowed to dry at 25°C for approximately 3 hours in a class III biological safety cabinet.

Tomato inoculation. Mature tomatoes (<24 hours after harvest) were obtained from a local farmer's market in Gainesville, Florida on the day of experimentation (Appendix 1). Non-ripe representative tomatoes (pinks to green) were obtained from a local grower for extended persistance trials. Waxed tomatoes were obtained from a local grocery store on the day of the experiment. The stem scars (1.0-1.5 cm^2), intact surfaces (0.7-1.0 cm^2), and pinpricked surfaces (0.7-1.0 cm^2) were selected and marked for inoculation by circumscribing the selected area with a permanent marker. Pin pricked surfaces were prepared by piercing with a sterile 25-gauge needle to a depth of approximately 3mm. Twenty-five microliters of inoculum suspension (deionized water or sewage carrier) was placed on intact surfaces, stem scars, and pin pricked surfaces. The inoculated tomatoes were air-dried at 25°C for approximately 3 hours in a class III biological safety cabinet.

Lettuce preparation and inoculation. The entire heads (except inedible base) of romaine lettuce were processed into segments resembling a typical salad size serving portion (Appendix 1). The outer approximate two thirds of the leaves were removed from the head of lettuce and were further processed and categorized into midrib and leaf tip portions. The midrib portions were approximately 3 x 6cm and were taken from the centermost portion of the leaf which included the main vein of the leaf. The remaining leaf portions were further cut into approximately 6 x 6cm portions. The interior one third of the head of the lettuce was categorized as the heart, and was cut into 3 x 6cm portions. The lettuce portions were segregated by category for localization studies. Samples to be subjected to treatments and persistence studies were prepared as above, but entire heads of lettuce were processed and mixed together. All samples were inoculated by placing 100g of processed lettuce into sterile stomacher bags (380 x 508cm Seward Scientific, United Kingdom). Each 100gm sample had 3.0ml of the inoculum cocktail sprinkled across the lettuce. The bag was folded and shaken to distribute the inoculum. The inoculated samples were then held at 4°C overnight (at least 18 hours) to allow complete attachment of the challenge organisms (Takeuchi and Frank, 2000).

Cantaloupe preparation and inoculation. Cantaloupes were obtained from a local grocery store in Gainesville, Florida on the day of the experiment (Appendix 1). For the localization studies, inocula of the various bacteria and viruses were added to a 2L beaker of deionized water to achieve a concentration of 10^7 PFU or CFU/ml. The melons were immersed in the seeded water for two minutes with constant manual agitation. The fruit was then removed from the inoculum and allowed to dry at 25°C in a laminar flow hood for approximately 18 hours. For disinfection and persistence trials, 1.5cm^2 areas were marked on the surfaces of the melons with a permanent marker, including blossom scars and surface scars. Fifty microliters of inoculum suspension were placed on the marked area, and dried approximately 18 hours prior to treatment or processing.

Microorganism elution from strawberries, tomatoes, and cantaloupe. The method of Lukasik et al. (2000) was utilized to elute the challenge organisms from strawberries, tomatoes and cantaloupe. At each sampling point or after treatments, individual strawberries were placed in sterile 250ml sample bags containing 30ml phosphate-buffered saline (PBS) with 0.1% (w/v) Tween 80 elution buffer (PBS-Tween), pH 7.4. Inoculated sample regions (intact surface, stem scars, or pin pricked surface) of each sample tomato were removed with an ethanol-sanitized scalpel and placed in sterile centrifuge tubes containing 5.0ml PBS-Tween. Inoculated sample regions of cantaloupes were also excised with an ethanol-sanitized scalpel and placed in sterile centrifuge tubes containing

7 0ml of PBS-Tween The samples were then subjected to rotary shaking at 60 rpm (Red Rotor Hoefer Scientific, San Francisco, CA) for 25 minutes at 36°C

Lettuce elution method evaluation Two physical elution methods were evaluated, homogenization on high setting for two minutes using a stomacher (Seward Scientific), or rotary shaking at 60 rpm (Red Rotor Hoefer Scientific) for 25 minutes at 36°C Twenty (20) grams of inoculated midrib or leaf tip portions were placed into sterile 250ml sample bags Eighty (80) milliliters of PBS-Tween were added to each sample bag Five samples from each category were subjected to the differing elution methods The trials were repeated twice and the resulting means of recovered organisms combined

Viral enumeration The bacteriophages in this study were enumerated as PFU/ml using mid log cultures of their respective hosts and a soft agar overlay technique (Snustad and Dean 1971) Poliovirus was assayed on BGM cells utilizing the agar overlay method reported by Smith and Gerba (1982)

Bacterial enumeration The *S* Montevideo and *E. coli* O157 H7 eluted from the samples were enumerated by spread plating samples on TSA-rif supplemented with 0 6 g/L of ferric ammonium citrate (Sigma Chemical Corp) and 6 6 g/L of sodium thiosulfate (Sigma Chemical Corp) (TSA rif w/supplements) The addition of the antibiotic rifampicin reduces the background microflora of the samples while the sodium thiosulfate and ferric ammonium citrate allows the bacterial colonies to be distinguished from each other (Lukasik et al , 2000) The plates were inverted and incubated at 35-37°C and counted within 24 hours

Chemical and non-chemical treatments Chlorine solutions were prepared immediately prior to application using household bleach (5.25% sodium hypochlorite, Clorox Co , Oakland, CA) and concentrations were monitored with a HACH Free Chlorine Kit (HACH Co , Loveland CO). The free chlorine levels in the test solutions evaluated include 50, 100 200, and 300ppm The chlorine test solutions were adjusted to a final pH of 7 3 by the addition of 0 1 molar hydrochloric acid prior to the treatment of strawberries, tomatoes and cantaloupe Lettuce samples were challenged with chlorine solutions adjusted to pH 7 3 and with unadjusted pH levels The effectiveness of the commercial stabilized chlorine dioxide compound Carnebon 200® (International Dioxide Inc, Clark NJ) was also evaluated The product was prepared according to label instructions and applied at 100ppm and monitored using a BCI Test Kit (Biocide International Norman OK) Tap water (0 03ppm free chlorine, pH 6 5) was also included as a treatment for strawberries, tomatoes, and cantaloupe Tap water treatment served as a control for lettuce samples All treatment solutions were prepared and applied at 43°C and all exposures to treatment solutions were for 2 minutes with constant agitation Treatments were administered by placing 5 of the inoculated tomatoes or strawberries into 2L of treatment solution Lettuce treatments were conducted by placing 100gms of the inoculated sample into 2L of treatment solutions. Treatments were applied to individual melons by immersion into 2L of treatment solution. After two minutes of exposure to treatment solutions, the produce samples were removed and placed into 2L of neutralizing solution (deionized water containing 0.05% (w/v) sodium thiosulfate) for 45 seconds, then removed and allow to drip dry briefly. The treated samples were then processed and eluted as described above.

Persistence of challenge organisms. Produce samples were prepared and inoculated utilizing either a deionized water or filtered wastewater carrier and stored at 4, 10 or 25°C. At predetermined time intervals, samples from each category were eluted, enumerated, and the data plotted as CFU or PFU/ml recovered over time.

Statistical analysis. Eluted populations of the challenge organisms were subjected to Student's paired t test and analysis of variance to determine significant differences ($p < 0.05$) between controls and treatments and between treatments. Four samples from each treatment category were eluted, the trials repeated twice and resulting means for number of organisms combined and reported. Persistence data presented is a combined $\log_{10}$ means of two replicate trials, each was composed of 4 samples at each time point. All samples were plated in duplicate.

CHAPTER IV
RESULTS

The objectives of this study included the evaluation of the effectiveness of chemical and non-chemical disinfection treatments on challenge organisms applied to the surfaces of strawberries, tomatoes, fresh-cut lettuce, and cantaloupe. The persistence of the challenge bacteria and viruses was also explored on these products under various storage conditions. In addition, strains of *E. coli O157:H7* and *Salmonella* Spp. were evaluated as challenge organisms using growth curves and attachment responses as criteria. Elution techniques were evaluated for the processing of inoculated lettuce samples. Localization preferences of the challenge bacteria when applied to lettuce and cantaloupes were also examined. These findings will be presented by produce type in this chapter.

STRAWBERRIES

Disinfection treatments. The reduction of the challenge bacteria and viruses are presented in Table 1. All treatments, including tap water, demonstrated significant reduction in the challenge organisms when compared to the control. Controls were inoculated samples that were *not* subjected to treatments. No significantly different reductions in any of the test organisms were observed between treatments with 50 and 100ppm chlorine solutions. Both bacteria strains were reduced in greater numbers by the 200ppm chlorine solution than by the 300ppm solution. The stabilized chlorine dioxide product demonstrated the highest reductions in *S*. Montevideo populations, with a 2.20 $\log_{10}$ reduction. Tap water was the least effective of the treatments examined on removing the seeded bacteria. Tap water did remove a substantial portion of the viral population, with 2.11, 1.19, and 1.03 $\log_{10}$ reductions obtained for Phi-X, MS2, and polio viruses, respectively. Two-hundred (200) ppm chlorine provided the highest reductions to all the challenge organisms of all the treatments evaluated, with the exception of *S*. Montevideo.

Table 1 Recovery of inoculated challenge organisms from strawberries in response to chemical and non-chemical treatments

	E. coli O157:H7	*S.* Montevideo	PRD-1	Phi-X 174	MS2	PV-1
Control	6.09* a	6.08 a	5.57 e	6.31 e	5.30 a	4.11 e
Chlorine 50ppm**	4.63 b c (1.46)	4.74 b,d (1.34)	3.51b d (2.06)	4.45 b (1.86)	3.13b d (2.17)	2.45b d (1.66)
Chlorine 100ppm	4.70 c (1.39)	4.66 d (1.42)	3.65b d (1.92)	4.38 b (1.93)	3.60 d (1.70)	2.70b e (1.41)
Chlorine 200ppm	4.13 d (1.96)	4.03 c (2.05)	2.77 d (2.80)	3.49 c (2.82)	2.72b d (2.58)	<1.0** d (>3.0)
Chlorine 300ppm	4.45 b d (1.64)	4.33 c (1.75)	2.95b d (2.62)	3.51 c (2.80)	2.79 b (2.51)	2.00d e (2.11)
Tap water	5.39 e (.70)	4.80 b d (1.28)	4.71 b (.86)	4.20 d (2.11)	4.11 c (1.19)	3.08 c (1.03)
Carnebon 200® (100ppm)	4.36 c (1.73)	3.88 b d (2.20)	4.02 c (1.55)	4.63 b (1.68)	5.19 e (2.95)	3.11 b (1.00)

*Values are presented as log$_{10}$ units with log$_{10}$ reduction in parentheses. Values in the same column with the same letters are not significantly different ($p<0.05$)

**Strawberries were immersed in deionized water containing approximately 10^7 challenge organisms, and air-dried for 3 hours prior to treatment. Control consists of 4 inoculated strawberries eluted after 3 hours with no treatment applied. Data presented represents replicate trials consisting of 4 strawberries subjected to each treatment, with the means of recovered organisms combined.

Persistence of challenge organisms. The persistence of bacteria and viruses inoculated onto strawberries via a deionized water carrier and stored at 4°C are presented in Figures 1 and 2. Both bacteria demonstrated greater than a two-log$_{10}$ reduction over the course of the study (Figure 1). As seen in Figure 2, viruses persisted well on the strawberries throughout the study, and maintained relatively high concentration post inoculation. Challenge organisms inoculated onto strawberries via a filtered sewage medium are presented in

Figures 2 and 3. The *S.* Montevideo populations were reduced greater than two $\log_{10}$ units by the 9th day, whereas *E. coli* O157:H7 was only reduced approximately one $\log_{10}$. The viruses inoculated via the sewage medium did not survive as well, and were reduced approximately 3 $\log_{10}$. Recoverable numbers were obtained for all challenge organisms after 9 days of storage at 4°C.

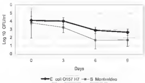

Figure 1. Bacteria persistence on strawberries inoculated with a deionized water carrier

*Strawberries were immersed in deionized water containing approximately 10^7 challenge bacteria in a deionized water carrier, and air-dried for 3 hours prior to initial sample processing and storage at 4°C. Data presented represents replicate trials consisting of 4 strawberries eluted at each time point, with the means of recovered organisms combined.

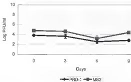

Figure 2 Viral persistence on strawberries inoculated with a deionized water carrier

*Strawberries were immersed in deionized water containing approximately 10^7 challenge viruses, and air-dried for 3 hours prior to initial sample processing and storage at 4°C Data presented represents replicate trials consisting of 4 strawberries eluted at each time point, with the means of recovered organisms combined

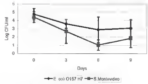

Figure 3 Bacteria persistence on strawberries inoculated with a sewage carrier

*Strawberries were immersed in filtered sewage containing approximately 10^7 challenge bacteria and air-dried for 3 hours prior to initial sample processing and storage at 4°C. Data presented represents replicate trials consisting of 4 strawberries eluted at each time point, with the means of recovered organisms combined

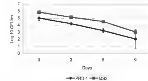

Figure 4 Viral persistence on strawberries inoculated with a sewage carrier

*Strawberries were immersed in filtered sewage containing approximately 10^7 challenge viruses in a deionized water carrier and air-dried for 3 hours prior to initial sample processing and storage at 4°C Data presented represents replicate trials consisting of 4 strawberries eluted at each time point, with the means of recovered organisms combined

TOMATOES

Growth curves. Prior to attachment studies of the challenge organisms, the growth patterns of the bacteria were determined. Figure 5 presents the results of an 18-hour growth study of the five strains of *E. coli O157:H7* examined. The strains all grew at a similar rate, although a spike in the curve was observed at hour 9. There was no significant difference in any of the populations found at hour 9 or any of the time points ($p<0.05$). A similar growth curve was produced for the *Salmonella* Spp. examined as presented in Figure 6. The *Salmonella* strains examined demonstrated no significant differences in mean populations at any time point, although a slight spike in the growth curve occurred at hour 15.

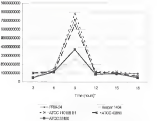

Figure 5. Eighteen-hour growth curve for E. coli O157:H7 strains

* One hundred (100) microliters of 18 hour TSB cultures of the respective strains were inoculated into 5ml of TSB and incubated at 36°C on a rotary shaker for 18 hours. Each strain was grown in triplicate, and at each time point samples were spread plated on TSA rif in duplicate. The mean populations of the respective strains were combined and plotted over time. No significant difference between mean populations of strains were found between strains at any time point examined. ($P<0.05$)

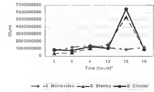

Figure 6 Eighteen hour growth curves for *Salmonella* strains

* One hundred (100) microliters of 18 hour TSB cultures of the respective strains were inoculated into 5ml of TSB and incubated at 36°C on a rotary shaker for 18 hours Each strain was grown in triplicate and at each time point samples were spread plated on TSA-nf in duplicate The mean populations of the respective strains were combined and plotted over time No significant difference between mean populations of strains were found between the strains at any time point examined ($P<0.05$)

Attachment and recovery. The inoculum concentration and populations recovered for the five strains of *E. coli O157:H7* examined reported as CFU/ml recovered from the tomato stem scars are presented in Table 2. The percent reduction of the initial populations ranged from 99.1% to 99.7% with no significant differences between the recovered populations. Table 3 presents the *Salmonella* Spp. examined for attachment performance traits. The *Salmonella* Spp. were reduced in similar amounts after drying, ranging from 99.6 to 99.8% reductions obtained from inoculation to recovery.

Table 2. Inoculation and recovery of *E. coli O157:H7* strains on tomato stem scar surfaces

Strain	Inoculm level	Recovery	Percent Reduction
110195*	9.5×10^7	3.0×10^5	99.7%
35150	3×10^7	4.6×10^4	99.8%
43888	1.2×10^8	5×10^5	99.6%
1404	8.5×10^7	6×10^5	99.1%
FRIK 24	3.4×10^8	1.6×10^6	99.5%

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of each of the *E. coli O157:H7* strains evaluated, and air-dried for 3 hours prior to elution. Data presented represents replicate trials consisting of 4 tomatoes inoculated with an individual strain, with the means of recovered bacteria combined.

Table 3 Inoculation and recovery of *Salmonella* strains on tomato stem scar surfaces

Strain	Inoculm level	Recovery	Percent Reduction
S. Montevideo*	2.1 x 10^7	7.5 x 10^{4**}	99.6%
S. chester	2.7 x 10^7	4.3 x 10^4	99.8%
S. stanley	1.1 x 10^7	3.4 x 10^4	99.7%

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of each of the *Salmonella* Spp. evaluated, and air-dried for 3 hours prior to elution. Data presented represents replicate trials consisting of 4 tomatoes inoculated with an individual strain, with the means of recovered bacteria combined

**No significant difference was obtained between recovered populations of challenge strains ($p<0.05$)

Disinfection treatments of stem scars. Both *E. coli O157:H7* and *S.* Montevideo populations subjected to immersion in 50ppm chlorine solutions (43°C) were not recovered in amounts significantly different from the controls (Table 4). Controls were inoculated samples that were not subjected to treatments. Tap water treatments did reduce *E. coli O157:H7* population significantly, but not the *S.* Montevideo populations. The responses by the *E. coli O157:H7* to treatments with 50, 100, and 300ppm chlorine were similar to each other, with less than a 1 $\log_{10}$ reduction for any of these treatments. A 1.94 $\log_{10}$ reduction in the *E. coli O157:H7* population was observed for the 200ppm solution and the chlorine dioxide product produced a .93 $\log_{10}$ reduction. *S.* Montevideo populations were reduced the greatest by 200ppm chlorine (2.10 $\log_{10}$). The phage PRD-1 was only reduced 1.22 $\log_{10}$ CFU/ml by 200ppm chlorine, the highest reduction demonstrated by any of the treatments evaluated.

Treatments with 300ppm chlorine produced the greatest amount of reduction in Phi-X populations (1.66 $\log_{10}$), but this treatment was not significantly different than 100 and 200ppm. All the treatments applied to the MS2 challenge organisms, including tap water, produced greater than a 1 $\log_{10}$ reduction, with the exception of 50ppm chlorine.

Table 4 Recovery of test inoculated challenge organisms from tomato stem scars in response to chemical and non-chemical treatments

	E. coli O157:H7	*S.* Montevideo	PRD-1	Phi-X 174	MS2
Control*	4.79 a**	3.98 a	3.75 a b	3.88 a	5.76 a
Chlorine 50ppm	4.49 a b (.30)	3.24 a, d (.74)	3.30 a b (.45)	3.53 a b (.35)	5.24 b (.52)
Chlorine 100ppm	4.08 b c (.71)	2.68 b (1.30)	3.52 a b (.23)	2.30 b (1.58)	3.15 c,e (2.61)
Chlorine 200ppm	2.85 c,d (1.94)	1.88 b,c (2.10)	2.53 a (1.22)	2.30 b (1.58)	3.48 c,e (2.28)
Chlorine 300ppm	4.14 b (.65)	2.97 b c (1.01)	3.64 a b (.11)	2.02 b (1.88)	2.84 c (2.92)
Tap water	4.41 b (.38)	3.55 a (.43)	3.14 a b (.61)	3.27a (.61)	4.08 d (1.68)
Camabon 200 (100ppm)	3.86 b d (.93)	3.21 b d (.77)	3.40 b (.35)	3.09 b (.79)	3.94 a d (1.82)

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of challenge organisms and air-dried for 3 hours prior to treatment or elution. Controls were inoculated samples eluted with no treatment application. Treatment was applied by immersing the inoculated tomatoes in the treatment solution for two minutes, followed by immersion in a neutralizing solution prior to elution. All treatment solutions were applied at 43°C. The pH of chlorine solutions evaluated were all 7.3. Data presented represents replicate trials consisting of 4 tomatoes subjected to each treatment, with the means of recovered bacteria combined. Values are presented as $\log_{10}$ CFU or PFU/ml with $\log_{10}$ reduction in parentheses.

**Values in the same column with the same letters are not significantly different ($p<0.05$)

Persistence of challenge organisms on stem scars inoculated with sewage carrier stored at 4°C. As seen in Figure 7, both *E. coli O157:H7* and *S.* Montevideo populations declined more than 2 $\log_{10}$ units after three days. *E. coli O157:H7* populations declined an additional $\log_{10}$ unit by day 16, but the *S.* Montevideo populations remained stationary. Figure 8 represents the persistence of the challenge viruses, with recoverable concentrations obtained after 16 days. The PRD-1 and MS2 phages each declined approximately 3 $\log_{10}$ units over the course of the study, while the polio virus declined approximately 2 $\log_{10}$ units after 16 days.

Persistence of challenge organisms on stem scars inoculated with sewage carrier stored at 25°C. The bacteria populations dropped approximately 5 $\log_{10}$ units one day after inoculation (Figure 9). Eight days post inoculation, both bacteria had increased 2 $\log_{10}$ units but the populations declined to below detectable limits (<1 $\log_{10}$) by day 16. The MS2 and PRD-1 phages each declined at least 3 $\log_{10}$ units one day after inoculation, but were both recovered after 16 days (Figure 10). Polio virus populations dropped only slightly over 1 $\log_{10}$ unit after one day of storage, but were below detectable limits at day 16.

Persistence of challenge organisms on stem scars inoculated with deionized water carrier stored at 4°C. The bacteria challenge organisms both declined more than 2 $\log_{10}$ units one day after inoculation (Figure 11). At day 13, the *E. coli O157:H7* populations had increased 1 $\log_{10}$ unit from the levels measured at day one, whereas *S.* Montevideo populations were below detectable limits. PRD-1 remained relatively stable over the 13 day storage declining in population just over 1 $\log_{10}$ unit from day 1 (Figure 12). MS2 declined at least 4 $\log_{10}$ units over the course of the study.

Persistence of challenge organisms on stem scars inoculated with deionized water carrier stored at 25°C. As seen in Figure 13, both *S.* Montevideo and *E. coli O157:H7* populations declined approximately 4 $\log_{10}$ units by day 6, but were detected on samples processed 13 days post inoculation. Viral challenge organisms inoculated with a water carrier declined below detectable limits by day 13 (Figure 14). All three viruses were present at detectable limits at day 5, and PRD1 was detected until day 9.

Persistence of bacteria on stem scars of waxed and un-waxed tomatoes inoculated with deionized water carrier stored at 10°C. Figure 15 presents persistence data for *S.* Montevideo and *E. coli O157:H7* on the stem scars of waxed and un-waxed tomatoes. Populations of both challenge organisms persisted on both waxed and un-waxed stem scars 21 days post inoculation when held at 10°C. *S.* Montevideo levels actually increased on both waxed and un-waxed tomatoes, while the *E. coli O157:H7* decreased over the course of the study. This increase in population may possibly be attributed to the vegetable oil coating present on the surface of the tomatoes, which the *S.* Montevideo populations may be able to utilize as a source of nutrients.

Persistence of bacteria on puncture wounds of waxed and un-waxed tomatoes inoculated with deionized water carrier stored at 10°C. The *S.* Montevideo populations applied to puncture wounds of both waxed and un-waxed tomatoes increased over the 21day study (Figure 16). *E. coli O157:H7* declined on both tomato types but was recovered 21 days post inoculation. The increase in the standard deviation of the sample populations (error bars) are the result of the influence of some samples containing high recoverable numbers of challenge organisms while other samples were below the detectable limits of 10 CFU/ml.

Persistence of bacteria on intact surfaces of waxed and un-waxed tomatoes inoculated with deionized water carrier stored at 10°C. Challenge organism populations declined below detectable limits 7 days post-inoculation on waxed and unwaxed surfaces as seen in Figure 17. However detectable amounts of *S.* Montevideo and *E. coli O157:H7* were recovered at day 21 from the surfaces of waxed tomatoes.

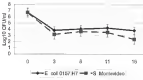

Figure 7 Bacteria persistence on tomato stem scars inoculated with sewage carrier and stored at 4°C

*Tomato stem scars were inoculated with filtered sewage containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 4°C. Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

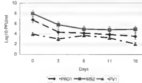

Figure 8 Viral persistence on tomato stem scars inoculated with sewage carrier and stored at 4°C

*Tomato stem scars were inoculated with filtered sewage containing approximately 10^7 of challenge viruses and air-dried for 3 hours prior to initial sampling and storage at 4°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point with the means of recovered viruses combined

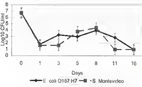

Figure 9 Bacteria persistence on tomato stem scars inoculated with sewage carrier and stored at 25°C

*Tomato stem scars were inoculated with filtered sewage containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 25°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point with the means of recovered bacteria combined

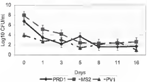

Figure 10 Viral persistence on tomato stem scars inoculated with sewage carrier and stored at 25°C

*Tomato stem scars were inoculated with filtered sewage containing approximately 10^7 of challenge viruses and air-dried for 3 hours prior to initial sampling and storage at 25°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered viruses combined

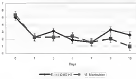

Figure 11 Bacteria persistence on tomato stem scars inoculated with deionized water carrier and stored at 4°C

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 4°C. Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

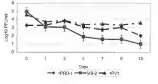

Figure 12 Viral persistence on tomato stem scars inoculated with deionized water carrier and stored at 4°C

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of challenge viruses and air-dried for 3 hours prior to initial sampling and storage at 4°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered viruses combined

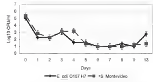

Figure 13 Bacteria persistence on tomato stem scars inoculated with deionized water carrier and stored at 25°C

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 25°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

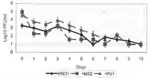

Figure 14 Viral persistence on tomato stem scars inoculated with deionized water carrier and stored at 25°C

*Tomato stem scars were inoculated with deionized water containing approximately 10^7 of challenge viruses and air-dried for 3 hours prior to initial sampling and storage at 25°C. Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered viruses combined

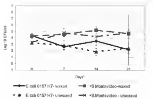

Figure 15 Bacteria persistence on stem scars of waxed and un-waxed tomatoes stored at 10°C

*Tomato stem scars of waxed and un-waxed tomatoes were inoculated with deionized water containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 10°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

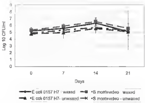

Figure 16 Bacteria persistance on puncture wounds applied to surfaces of waxed and un-waxed tomatoes stored at 10°C

*Puncture wounds were inflicted to the intact surfaces of waxed and un-waxed tomatoes. These puncture wounds were inoculated with deionized water containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 10°C. Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

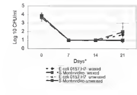

Figure 17 Bacteria persistence on intact surfaces of waxed and un-waxed tomatoes stored at 10°C

*Intact surfaces of waxed and un-waxed tomatoes were inoculated with deionized water containing approximately 10^7 of challenge bacteria and air-dried for 3 hours prior to initial sampling and storage at 10°C Data presented represents replicate trials consisting of 4 tomatoes sampled at each time point, with the means of recovered bacteria combined

LETTUCE

Physical elution. Mechanical methods to elute seeded bacteria and virus from lettuce were compared (Table 5). Rotary shaking and stomaching methods released comparable amounts of *E. coli* O157:H7 bacteria and Phi-X and MS2 viruses from midrib regions. Significantly higher numbers of PRD-1 were recovered using the stomaching methods on the midrib samples when compared to the shaking method. Rotary shaking resulted in significantly higher counts of *E. coli* O157:H7 than did stomaching the leaf tip samples. Similar amounts of *S.* Montevideo were eluted using the rotary shaking method. There were no significant differences observed in populations recovered between the shaking and stomaching methods for *S.* Montevideo, PRD-1, Phi-X, and MS2 viruses inoculated on the leaf tip samples.

Localization. The recovery of seeded bacteria and viruses from various lettuce regions, after overnight storage at 4°C is shown in Table 6. The bacteriophages PRD-1 and Phi-X were recovered in similar amounts from the midrib, leaf tip, and heart portions. Similar amounts of MS2 were recovered from midrib and leaf tips, and there were no significant differences in amounts recovered between the leaf tip and heart samples. Higher amounts of MS2 were, however, recovered from the midrib areas of the inoculated lettuce. Both *E. coli* O157:H7 and *S.* Montevideo were eluted in significantly lower quantities from the heart region.

Table 5 Recovery of challenge organisms inoculated onto midrib leaf tip and heart portions of romaine lettuce

Location	E. coli O157 H7	S. Montevideo	PRD-1	Phi-X 174	MS2
Inoculum	6.8 x 10^{7}a**	4.3 x 10^{8}a	8.3 x 10^{7}e	2.4 x 10^{7}e	9.2 x 10^{8}e
Midrib*	1.1 x 10^{7}b	9.2 x 10^{6}b	8.9 x 10^{3}b	3.3 x 10^{5}b	8.4 x 10^{4}b
Leaf tip	9.6 x 10^{6}b	8.3 x 10^{6}b	7.7 x 10^{3}b	1.5 x 10^{5}b	4.0 x 10^{4}b c
Heart	1.7 x 10^{6}c	2.4 x 10^{5}c	6.9 x 10^{3}b	2.1 x 10^{3}b	2.8 x 10^{4}c

*Lettuce samples were segregated by category and subdivided into 100gm samples which were inoculated with 3.0mls of inoculum and held at 4°C 18 hours prior to elution Data presented represents replicate trials consisting of 5 20gm samples of each category, with the means of recovered organisms combined, reported as CFU or PFU/ml

**Values in the same column with the same letters are not significantly different ($p<0.05$)

Table 6 Recovery of challenge organisms from midrib leaf-tip and heart portions of romaine lettuce following differing elution methods

Location (recovery method)	*E coli* O157 H7	*S* Montevideo	PRD-1	Phi-X 174	MS2
Inoculum*	8.8 x 10^8a**	4.3 x 10^8a	5.3 x 10^7a	2.4 x 10^7a	9.2 x 10^6a
Midrib (stomaching)	1.3 x 10^7b	2.0 x 10^7 b	1.5 x 10^5b	3.0 x 10^5b	7.8 x 10^4b
Midrib (shaking)	1.3 x 10^7 b	9.8 x 10^6 c	9.2 x 10^5c	3.2 x 10^5b	8.9 x 10^4b
Leaf tip (stomaching)	3.2 x 10^6 c	8.4 x 10^6 c	1.0 x 10^5c	1.9 x 10^5c d	5.9 x 10^4b c
Leaf tip (shaking)	1.1 x 10^7 b	8.4 x 10^6 c	8.1 x 10^5c	1.7x 10^5b d	3.7 x 10^4 c

*Lettuce samples were segregated by category and subdivided into 100gm samples, which were inoculated with 3.0mls of inoculum, and held at 4°C 18 hours prior to treatment. Data presented represents replicate trials consisting of 5 20gm samples of each category subjected to the respective treatments, with the means of recovered organisms combined reported as CFU or PFU/ml

**Values in the same column with the same letters are not significantly different ($p<0.05$)

Disinfection treatments The $\log_{10}$ reduction of the challenge bacteria and viruses are presented in Table 7 which presents data collected from samples treated with the commercial chlorine dioxide product Carnebon 200®, and free chlorine solutions prepared without adjusting the pH. All treatments demonstrated significant reduction in the challenge organisms when compared to the control. The control was 5 inoculated 20gm samples which were subjected to 2 minutes of 43°C tap water treatments applied followed by immersion in neutralizing solution prior to elution. No difference in responses to 50 and

100ppm chlorine treatments were observed for all bacteria and viruses with the exception of polio virus One log$_{10}$ or greater reduction in the challenge bacteria was only observed with 300ppm chlorine and the commercial chlorine dioxide product for *S* Montevideo MS2 populations were reduced 1 3 log$_{10}$ with the chlorine dioxide product at 100ppm. The polio virus populations were reduced in excess of one log for 100, 200 and 300ppm chlorine, but there was no significant difference in response to treatments with 50ppm chlorine and 100ppm chlorine dioxide Table 8 presents the comparison of free chlorine treatments with the pH adjusted to 7 3 or unadjusted solutions The unadjusted solution concentrations and their resulting equilibrated pH values are as follows, 50ppm 8 8, 100ppm 9 2 200ppm 6 5 and 300ppm 9 8 Polio virus did not appear to react differently to the challenge solutions with differing pH at similar free chlorine concentrations through the complete range of treatments Bacteriophages in general were reduced in comparable amounts when treated with chlorine solutions with different pH levels The bacterial pathogens however demonstrated a greater sensitivity to free chlorine solutions with the pH adjusted to 7 3 at concentrations greater than 50ppm

Table 7 Recovery of inoculated challenge organisms from fresh-cut lettuce in response to chemical treatments

	E. coli O157:H7	*S.* Montevideo	PRD-1	Phi-X	MS2	PV-1
Controls*	5.66 a**	5.84 a	6.89 a	5.69 a	6.36 a	5.00 a
Chlorine 50ppm pH 6.8	5.35 b (.31)	4.85 b c (.79)	6.70 b (.19)	5.32 b (.37)	6.09 b (.27)	4.14 b (.86)
Chlorine 100ppm pH 9.2	5.41 b (.25)	4.60 c (.74)	6.65 b (.24)	5.25 b,c (.44)	5.93 b (.43)	3.68 c (1.32)
Chlorine 200ppm pH 9.5	5.26 b c (.40)	4.67 b,e (.97)	6.55b d (.34)	5.19 c (.50)	5.85 b (.51)	3.81 c (1.19)
Chlorine 300ppm pH 9.8	4.94 c (.72)	4.41 d (1.23)	6.17 c (.72)	4.86 d (.83)	5.70 b (.66)	3.25 d (1.75)
Carnebon 200 (100ppm) pH 2.3	5.00 c (.66)	4.61 e (1.03)	6.47 d (.42)	5.39 b (.30)	5.14 c (1.22)	4.12 b (.88)

*Fresh-cut lettuce samples were divided into 100gm samples, which were inoculated with 3.0mls of inoculum containing approximately 10^7 challenge organisms and held at 4°C 18 hours prior to treatment. Data presented represents replicate trials consisting of five 20gm samples subjected to the respective treatments with the means of recovered organisms combined. Control was 5 inoculated 20gm samples which were subjected to 2 minutes of 43°C tap water treatments applied followed by immersion in neutralizing solution prior to elution. The chlorine solutions were applied in as obtained during formulation without the addition of acid (unadjusted). Treatment was applied by immersing the 100gm samples of inoculated lettuce in the treatment solution for two minutes followed by immersion in a neutralizing solution prior to elution. All treatment solutions were applied at 43°C. The pH of chlorine solutions evaluated were not adjusted by the addition of any acidifying agents. Values are presented as $\log_{10}$ units with $\log_{10}$ reduction in parentheses.

** Data presented as $\log_{10}$ CFU or PFU/ml. Values in the same column with the same letters are not significantly different ($p<0.05$)

Table 8. Reduction of inoculated challenge organisms on fresh-cut lettuce in response to chemical treatments

	E. coli O157:H7	*S.* Montevideo	PRD-1	Phi-X	MS2	PV1
Control*	5.86 a**	5.79 a	6.83 a	5.80 a	7.07 a	4.93 a
50ppm						
pH 7.3	5.70 b	5.13 b	6.43 b,d,e	5.42 b,h	7.29 b	4.40 b
pH 8.8	5.12 c	4.89 b,d	6.56 b	5.37 b,d	6.31 c	4.10 b,d,f
100ppm						
pH 7.3	5.11 c	4.37 c,f	6.12 d,e	5.15 c,e,f	7.13 b	3.93 c,d,f
pH 9.2	5.19 c	4.82 b,d	6.44 e	5.15 c,e,f	6.18 d	3.66 c,g
200ppm						
pH 7.3	4.93 d	4.10 c	5.93 c	5.10 d,e,f	6.94 e	2.22 b,d,e,f,g
pH 9.5	5.21 c	4.80 d	6.36 d,e	5.22 b,e	6.11 d	3.76 d
300ppm						
pH 7.3	4.77 e	3.73 e	5.78 c	4.99 f	6.75 e	2.64 b,d,e,f,g
pH 9.8	4.69 d,e	4.48 f	5.89 c	4.84 g	5.96 d	3.18 e

*Fresh cut lettuce samples were divided into 100gm samples which were inoculated with 3.0mls of inoculum containing approximately 10^7 challenge organisms, and held at 4°C 18 hours prior to treatment. Data presented represents replicate trials consisting of five 20gm samples subjected to the respective treatments with the means of recovered organisms combined. Control was 5 inoculated 20gm samples which were subjected to 2 minutes of 43°C tap water treatments, followed by immersion in neutralizing solution prior to elution. The chlorine solutions were applied as is obtained during formulation without the addition of acid (unadjusted) or adjusted by the addition of hydrochloric acid to a final pH of 7.3. Treatment was applied by immersing the 100gm samples of inoculated lettuce in the treatment solution for two minutes, followed by immersion in a neutralizing solution prior to elution. All treatment solutions were applied at 43°C. The pH of chlorine solutions are indicated.

** Data presented as $\log_{10}$ CFU or PFU/ml. Values in the same column with the same letters are not significantly different ($p<0.05$)

Persistence of challenge organisms. The persistence of bacteria and viruses inoculated onto chopped lettuce stored and stored at 4°C are presented in Figures 18 and 19. The bacteria demonstrated a near two $\log_{10}$ reduction over the course of the study (Figure 18). As seen in Figure 19, viruses persisted on the lettuce throughout the study, dropping approximately 1 $\log_{10}$ reduction over the course of the storage study. Recoverable numbers of all challenge organisms were obtained after 10 days of storage at 4°C.

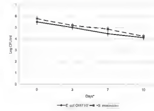

Figure 18. Bacteria persistence on fresh-cut romaine lettuce inoculated with deionized water carrier and stored at 4°C

*Fresh-cut lettuce samples were divided into 100gm samples, which were inoculated with 3.0mls of inoculum containing approximately 10^7 challenge organisms, and held at 4°C 4 hours prior to the initial sampling, and storage at 4°C. Data presented represents replicate trials consisting of five 20gm samples eluted at each time point, with the means of recovered bacteria combined

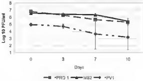

Figure 19 Viral persistence on fresh-cut romaine lettuce inoculated with deionized water carrier and stored at 4°C

*Fresh-cut lettuce samples were divided into 100gm samples which were inoculated with 3.0mls of inoculum containing approximately 10^7 challenge viruses, and held at 4°C 4 hours prior to the initial sampling, and storage at 4°C. Data presented represents replicate trials consisting of five 20gm samples eluted at each time point with the means of recovered viruses combined

CANTALOUPE

Localization The preferences for attachment to the various regions on cantaloupe by the challenge organisms were examined and the results presented in Table 9 The challenge bacteria both demonstrated a propensity for attachment to the stem scar region with significantly higher populations recovered from this area than the other areas examined The MS2 phage also was recovered from the stem scar region but not in amounts significantly different from the blossom scar area, where slightly higher concentrations were recovered

Table 9 Location of challenge organisms recovered from inoculated cantaloupe

Location	*E coli O157 H7*	*S* Montevideo	MS2
Blossom scar*	3 28 a**	2 02 a	4 66 a
Stem scar	4 91b	4 36 b	4 02 a,c
Top intact	3 05 a	2 02 a	3 31 b
Bottom intact	3 64 c	2 33 a	3 95 c

*Cantaloupe were immersed in deionized water containing approximately 10^7 challenge organisms and air-dried for 18 hours prior to sampling Data presented represents replicate trials consisting of 4 four melons with 4 separate samples excised from the top and bottom of the inoculated melon The stem scar and blossom scars were also removed from each melon and the combined means of the recovered organism reported by region Data reported as $\log_{10}$ CFU or PFU/ml

** Data presented as $\log_{10}$ CFU or PFU/ml Values in the same column with the same letters are not significantly different ($p<0 05$)

Disinfection treatments. The reductions in the populations of the challenge organisms are presented in Table 10. Tap water treatments did not produce significant reductions in the challenge bacteria populations with only 0.47 and 0.51 $\log_{10}$ reductions observed in *E. coli O157:H7* and *S.* Montevideo respectively. The bacteriophage populations were all recovered in significantly lower amounts than the control after the tap water treatments. The *E. coli O157:H7* and *S.* Montevideo populations responded similarly to the range of chlorine treatments, with no significant difference observed between treatments. The greatest reduction in the populations of PRD-1 and Phi-X populations was observed at 200ppm free chlorine, with 300ppm producing the highest $\log_{10}$ reductions in the MS2 populations. The chlorine dioxide product produced a greater than 2 $\log_{10}$ reduction in 4 of the 5 challenge organisms, with MS2 being reduced only 1.60 $\log_{10}$. The reductions of *E. coli O157:H7*, *S.* Montevideo, and PRD-1, however, were not significantly different than 50ppm free chlorine treatments. The Phi X and MS2 populations were reduced in amounts comparable to 100ppm free chlorine treatments by the chlorine dioxide treatment at 100ppm.

Table 10 Recovery of inoculated challenge organisms from cantaloupe in response to chemical treatments

Sample	*E coli O157 H7*	*S* Montevideo	PRD-1	Phi-X 174	MS2
Controls*	5.28 a**	5.07a	6.06 a	5.19 a	5.87 a
Chlorine 50ppm	2.69 b c (2.59)	2.31 b (2.76)	3.61 b e (2.26)	1.75 b (3.44)	4.16 b (1.72)
Chlorine 100ppm	1.42 b (3.86)	1.62 b (3.45)	3.55 b c (2.51)	1.97 b d (3.23)	4.04 c (1.83)
Chlorine 200ppm	1.62 b (3.65)	1.75 b (3.32)	2.94 c d (3.12)	<D L **	3.67 d (2.20)
Chlorine 300ppm	2.99 b c (2.29)	3.43 b (1.64)	3.36 b d (2.70)	1.50 b (3.69)	3.58 d (2.29)
Tap water	4.61 a (0.47)	4.56 a (0.51)	4.00 b (2.07)	2.94 c (2.25)	4.66 b c d (1.01)
Carnebon 200 (100ppm)	2.71 c (2.57)	2.53 b (2.54)	3.37 c d,e (2.69)	2.26 d (2.93)	4.06 c (1.80)

*Cantaloupe were inoculated by depositing 50µl of inoculum suspension containing approximately 10^7 challenge organisms onto pre-marked 1.5cm^2 locations on the surface of the melons, and air-dried for 18 hours prior to treatment. Treatment was applied by immersing the inoculated portion of the melon in the treatment solution for two minutes, followed by immersion in a neutralizing solution prior to elution. All treatment solutions were applied at 43°C. The pH of chlorine solutions evaluated were all 7.3. Data presented represents replicate trials consisting of 4 four melons per treatment, with 4 samples excised from each melon.

** Data presented as $\log_{10}$ CFU or PFU/ml. Values in the same column with the same letters are not significantly different (p<0.05)

Persistence of challenge organisms. The populations of bacteria and viruses recovered from the surfaces of cantaloupe stored at 4°C are presented in Figures 20 and 21. Both bacteria populations declined by nearly 2 $\log_{10}$ units by day 7, but were both recovered 10 days post-inoculation. *E. coli O157:H7* were recovered 30 days after inoculation, but no viable *S.* Montevideo were recovered. The MS2 and PRD-1 populations declined approximately 2 logs over the course of the 30-day storage trial, while the polio virus only declined by slightly more than one $\log_{10}$.

The bacteria populations declined rapidly on the surfaces of cantaloupe stored at 25°C, and both *E. coli O157:H7* and *S.* Montevideo were near or below detectable limits (10 CFU/ml) after 7 days (Figure 22). The challenge viruses only declined approximately one $\log_{10}$ PFU/ml over the course of the 10-day storage trial as seen in Figure 23.

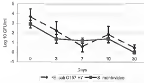

Figure 20 Bacteria persistence on cantaloupe surfaces inoculated with deionized water carrier and stored at 4°C

*Cantaloupes were inoculated by depositing 50µl of inoculum suspension containing approximately 10^7 challenge bacteria onto pre-marked 1.5cm^2 locations on the surface of the melons The samples were dried for 5 hours prior to initial sampling and storage at 4°C Data presented represents replicate trials each consisting of 2 melons per time point with 4 samples excised from each melon

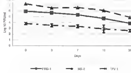

Figure 21 Viral persistence on cantaloupe surfaces inoculated with deionized water carrier and stored at 4°C

*Cantaloupes were inoculated by depositing 50µl of inoculum suspension containing approximately 10^7 challenge viruses onto pre-marked 1 5cm^2 locations on the surface of the melons The samples were dried for 5 hours prior to initial sampling and storage at 4°C Data presented represents replicate trials each consisting of 2 melons per time point with 4 samples excised from each melon Where no error bars visible standard deviation <0 2 log$_{10}$ CFU/ml.

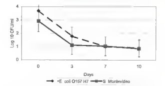

Figure 22. Bacteria persistence on cantaloupe surfaces inoculated with deionized water carrier and stored at 25°C

*Cantaloupes were inoculated by depositing 50µl of inoculum suspension containing approximately 10^7 challenge bacteria onto pre-marked 1.5cm^2 locations on the surface of the melons. The samples were dried for 5 hours prior to initial sampling, and storage at 25°C. Data presented represents replicate trials, each consisting of 2 melons per time point with 4 samples excised from each melon.

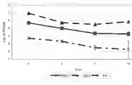

Figure 23 Viral persistence on cantaloupe surfaces inoculated with deionized water carrier and stored at 25°C

*Cantaloupes were inoculated by depositing 50µl of inoculum suspension containing approximately 10^7 challenge viruses onto pre-marked 1.5cm^2 locations on the surface of the melons. The samples were dried for 5 hours prior to initial sampling, and storage at 25°C. Data presented represents replicate trials, each consisting of 2 melons per time point with 4 samples excised from each melon. Where no error bars visible, standard deviation <0.2 $\log_{10}$ CFU/ml

CHAPTER V
DISCUSSION AND CONCLUSIONS

The major points to be addressed in this chapter include a discussion of challenge organism selection, the development of elution techniques, the localization of challenge organisms on the surfaces examined in this study, and the responses of the challenge organisms to water, chlorine, and chlorine dioxide treatments. The discussion of the persistence trials will address each produce type separately, followed by general conclusions and implications of this research study.

Challenge organisms. The selection of appropriate challenge organisms to evaluate a given variable can be one of the most difficult and often debated tasks to perform prior to beginning a research project. The organism of interest should be representative of similar strains and exhibit qualities similar to other organisms. The scientific literature reveals that microorganisms differ greatly in their response to disinfectants (Beuchat, 2000), and the selection of challenge organism appropriate for the parameters to be evaluated may vary to suit specific needs. Therefore the extrapolation of the data presented in this study to other produce items and under other conditions must be done with great care.

For this study five strains of *E coli O157 H7* and three *Salmonella* Spp were evaluated for growth patterns and responses to inoculation on and recovery from tomato stem scar surfaces. The strains of *E coli O157 H7* examined all performed similarly in both growth and attachment/recovery experiments The *Salmonella Spp* evaluated responded in a similar manner, with no significant differences in either growth or attachment and recovery criteria *S* Montevideo G4639 and *E coli O157 H7* (ATCC 110195-B1) both have previously been included in disinfection and persistence trials (Castillo et al., 1999 Lin and Wei 1997 Wei et al. 1995) These strains performed similarly to the other strains evaluated under both settings examined in this study and were selected as representative challenge organisms for the remainder of the project.

Lettuce elution technique development. Lukasik et al (2000) reported an elution technique to remove bacteria and viruses from the surface of inoculated tomatoes and strawberries. This technique was also applied to cantaloupe in this study but was not appropriate for the elution of challenge organisms from lettuce due to the differing structure of the produce items Two mechanical elution methods were evaluated to identify a physical process that would produce the most efficient elution of pathogens from lettuce Both shaking and stomaching have been utilized in previous studies to elute leafy produce (Beuchat 1999 Escudero et al 1999, Finn, and Upton 1997 Hao et al 1999) As seen in the presented results (Table 5) stomaching and shaking eluted similar amounts of bacteria from both midrib and leaf tip regions of the inoculated lettuce Rotary shaking and stomaching methods released comparable amounts of *E. coli* O157:H7 bacteria and Phi-X and MS2 viruses from midrib regions. Stomaching the midrib samples eluted higher numbers of PRD-1 than the shaking method. Shaking the leaf tips resulted in significantly higher counts of *E. coli* O157:H7 than did stomaching, and eluted similar amounts of *S.* Montevideo. There were no significant differences observed in populations recovered between the shaking and stomaching methods for *S.* Montevideo, PRD-1, Phi-X, and MS2 viruses inoculated on the leaf tip samples. Lukasik et al. (2000) also compared stomaching and rotary shaking techniques for tomatoes and strawberries, and found the shaking method eluted a comparable number of bacteria and twice as many viruses compared to stomaching. It has been previously indicated that aerosolization of samples can have antagonistic affects on viral agents (Berg et al. 1984). The degree of aerosolization the challenge viruses were subjected to during the elution process did not appear to adversely affect the recovery process. However, because the shaking method was considerably less physically disruptive for the lettuce samples and presents less potential for the aerosolization of the seeded viruses, while eluting similar amounts of seeded organisms, the shaking method of elution was selected as the most conservative elution method to apply to lettuce samples.

Localization. Lettuce was inoculated with challenge bacteria and selected viruses to midrib, leaf tip, and heart portions of romaine lettuce that were held at 4°C for 18 hours prior to sampling. The holding of the product at this temperature and length of time reportedly allows for the maximum attachment of the challenge organisms used in other studies of lettuce (Takeuchi and Frank, 2000). The phages Phi-X-174 and PRD1 were recovered in similar amounts from all three regions (Table 6). Similar amounts of MS2 were recovered from midrib and leaf tips, and there were no significant differences in populations recovered from the leaf tip and heart samples. Higher amounts of MS2 were, however, recovered from the midrib areas of the inoculated lettuce. *E. coli* O157:H7 and *S.* Montevideo were recovered in comparable amounts from the midrib and leaf tip samples, but in significantly lower amounts from the heart region of the romaine lettuce. Takeuchi and Frank (2000) reported that *E. coli* O157:H7 strain 994 attached preferentially to the cut edges of iceberg lettuce over intact leaves. Seo and Frank (1999) further indicated in their study that *E. coli* O157:H7 strain 932 penetrated iceberg lettuce tissue at the cut edges, and were likely to remain viable after disinfection treatments. The higher counts of bacteria recovered from the midrib and leaf tip portions of fresh-cut romaine lettuce in this study, therefore, are not surprising. The midrib presents increased cut surface area for challenge organism attachment and exposure to the vascular tissue of the leaf. The surface of an undamaged lettuce leave is covered with cutin that helps protect the interior tissue from desiccation (Romberger et al., 1993). This waxy, hydrophobic layer may facilitate the attachment of the bacteriophages as well as bacteria. PRD1 and MS2, for example, exhibit a propensity toward attachment to surfaces via hydrophobic interactions (Lukasik, 1998) and this may be a factor in their attachment to produce surfaces. The localization of viral agents on precut and minimally processed produce items, and further examination of the mechanisms utilized in the attachment and subsequent displacement techniques may warrant further research.

Cantaloupes were immersed in a cocktail of at least 10^8 CFU or PFU/ml challenge organisms and various locations on the melons were examined to determine where the organisms would attach. Melons were immersed in two liters of the cocktail solution and held at 25°C for 18 hours prior to elution. The MS2 phage was recovered in similar amounts from the stem scar and the blossom scar, with both regions providing acceptable surfaces for attachment. These regions may also have remaining deposits of plant sap or residual organic matter enhancing the ability for the challenge organisms to attach to these areas. Significantly higher amounts of *E. coli* O157:H7 and *S.* Montevideo were recovered from the stem scar areas than any of the other regions examined. The removal of the stem at harvest and the subsequent exposure of vascular tissue upon which introduced organisms may attach may help explain the quantity of challenge organisms recovered from this location. The are no published studies to date addressing the attachment of viruses to surfaces of produce, nor the evaluation of preferential attachment locations of inoculated bacteria on cantaloupe. Researchers have, however, demonstrated that cantaloupes immersed in inoculum of *S. stanley* may be transferred to the interior edible tissue during slicing (Ukuku and Sapers, 2000). Suslow et al. (2000) demonstrated that the intact rind of the cantaloupe is not an absolute barrier to artificially inoculated or indigenous bacteria, with *S* Typhimurium LT2 found to transfer 5mm below the rind Suslow et al. (2000) also reported that bacteria inoculated on the blossom scar and stem scar were occasionally detected in the interior flesh after storage The data presented in this study indicate that when immersed in an aqueous solution containing *E. coli* O157 H7, *S.* Montevideo and the model virus MS2 these challenge organisms could be recovered from the intact rind surfaces of cantaloupe However higher concentrations of these organisms attached to the stem scar and blossom scar regions of the cantaloupe This information, combined with the potential for these contaminants to be introduced to the interior edible tissue as demonstrated by Suslow et al (2000) and Ukuku and Sapers (2000) may contribute to the concern about the potential for cantaloupe to serve as a vehicle for the spread of foodborne illness Mohle-Boetani et al (1999) suggested that restaurant-cut cantaloupe may pose a greater risk than home-cut melons due the processing of multiple cantaloupes at a time, with one contaminated rind potentially contaminating the edible tissue of several fruit However the careful removal of these regions prior to slicing the fruit may help in reducing the risk of the introduction of contaminates into the interior of the fruit. Further research into such simple yet preventive measures may be appropriate

Water treatments Washing fruits and vegetables with potable water either immediately after harvest, prior to further processing or prior to consumption at the point of use is undoubtedly the most frequent treatment applied to produce Initial trials in this study included treatments with tap water of varying temperatures with 43°C water producing the greatest reductions in the challenge organisms from the surfaces of strawberries (data not shown) The results obtained from the temperature evaluation were similar to other researchers, who also report warmer temperatures removed more organisms from the surfaces of produce (Adams et al , 1989) Tap water was included as a treatment for tomato stem scars and did reduce *E coli O157 H7* and MS2 populations significantly but not the *S* Montevideo, PRD-1 or Phi-X populations in this study The 43°C tap water treatment produce statistically significant reductions in all the challenge organisms on strawberries Tap water also produced statistically significant reductions in all the challenge viruses on the surfaces of cantaloupe The attached bacteria however proved more resistant, with *E coli O157 H7* and *S.* Montevideo populations recovered after tap water treatments in amounts not significantly different from controls Ukuku and Sapers (2000) reported that washing with water did not reduce the attached populations of *Salmonella* in their study of inoculated lettuce similar to what was found in this study

It has been reported in the literature that washing produce items in hot water removes a portion of the pathogenic and spoilage organisms present (Adams et al , 1989) but scientific studies evaluating the efficacy of this simple and prevalent treatment are few (Beuchat, 2000) The findings of this study indicate that the effectiveness of potable water treatments vary with produce type and challenge organism. Further examinations of the effects of washing with potable water and the effectiveness of commercial washing operations may be necessary to determine if the resistance observed in the laboratory is similarly observed in commercial operations.

Chlorine treatments. Chlorine solutions are possibly one of the most common chemical disinfection treatments currently applied to produce. These solutions are frequently used at concentrations of 50-200ppm with short exposure times of 1-2 minutes (Beuchat, 2000). While this study also included concentrations of 300ppm, the 200ppm chlorine solution provided higher reductions in the challenge organisms than 300ppm with several of the produce types examined. Treatment with 200ppm chlorine produced the highest reductions in challenge bacteria on strawberries and tomatoes of all treatments evaluated. The bacteria populations on the surfaces of cantaloupes were reduced in the highest amounts by 100ppm chlorine. There was no statistical difference observed, however, between any of the chlorine solutions applied to cantaloupe. Previous researchers also found no significant difference between tomatoes inoculated with *S.* Montevideo and treated with 110 and 310ppm free chlorine solutions (Zhuang et al., 1995), as was seen in this study with 100 and 300ppm chlorine treatments on tomatoes and strawberries.

Lettuce proved to be the most difficult produce type to disinfect with the chlorine treatments. Chlorine solutions were applied to lettuce as they would be formulated at a retail food service establishment, home, or some commercial settings (i.e., the pH would not be decreased by the addition of an acid). No difference in responses to 50 and 100ppm chlorine treatments was observed for the challenge bacteria. While chlorine treatments did provide statistically significant reductions in bacterial populations when compared to the control, greater than one-log$_{10}$ reduction in bacterial populations was only achieved for *S.* Montevideo at 300ppm, while a 0.72 log$_{10}$ reduction in the *E. coli* O157:H7 populations was obtained at this concentration.

To further explore the effect of pH on the disinfection properties of the chlorine treatments, a range of free chlorine solutions were compared, with one set having the pH lowered to 7.3, the other unadjusted. The pH of the unadjusted solutions ranged from 8.8 to 9.8. This increase in pH is due to the inclusion of excess hydroxyl ions into the bleach solutions included to help stabilize the product against autocatalytic losses (White, 1992). While the viruses, in general, were reduced by similar amounts in response to the treatments with differing pH levels, there was a difference observed in the responses of the bacteria on the inoculated lettuce. Both *E. coli* O157:H7 and *S.* Montevideo populations were reduced at a greater degree using the solutions with a pH of 7.3 at concentrations above 50ppm. The reductions of the bacteria on lettuce presented in this study are only slightly higher than those reported by other researchers who used similar concentrations (200ppm free chlorine), combined with slightly longer exposure times (5 minutes) and lower treatment solution temperatures (22°C) and reported 0 7 and 1 0 log$_{10}$ reductions in *E. coli* O157 H7 populations on iceberg lettuce leaf surfaces and cut edges respectively (Seo and Frank 1999)

While the evaluation of various disinfection treatments of tomatoes artificially contaminated with Salmonella Spp (Wei et al , 1995 Zhuang and Beuchat 1996) and other pathogens (Beuchat and Brackett 1991) have been reported disinfection of surface associated viruses have not been reported in the scientific literature to date This study evaluated the response of selected reference viruses to treatments of chlorine solutions when attached to the surface of strawberries tomatoes lettuce, and cantaloupe

Greater reductions were observed in populations of PRD1 MS2 and polio virus treated with 50ppm chlorine than those obtained with 100ppm on inoculated strawberries Similar responses by bacteria have been reported by Escudero et al (1999) who found greater reductions in *Yersinia enterocolitica* populations on lettuce when treated with 25ppm chlorine compared to 100ppm solutions Similar seemingly contradictory reductions were observed as with the bacterial responses to 200 and 300ppm chlorine in some of the challenge viruses on strawberries, tomatoes and cantaloupe The MS2 populations on tomatoes and cantaloupe were reduced in greatest amounts by treatment with 300ppm free chlorine However these reductions were significantly different from those obtained with treatments at 200ppm

The viruses on the surfaces of lettuce responded quite differently from the bacteria challenge organisms to the chlorine treatments. MS2 was recovered after all chlorine treatments with no significant differences found between chlorine treatments. The polio virus populations were reduced in excess of one $\log_{10}$ for 100, 200, and 300ppm chlorine. This study, however, may be the first to demonstrate that bacteriophages and polio virus attached to lettuce tissue are quite resistant to disinfection with chlorine solutions. The response of polio virus and its similar response to chlorine solution of differing pH may support the need for additional research in the area of produce disinfection with emphasis placed on viral agents. The results obtained in this, and other studies evaluating disinfection treatments on lettuce, indicate that once bacterial pathogens and viruses are attached to lettuce, elimination of these organisms is difficult.

While reductions in excess of 2 $\log_{10}$ units were achieved by chlorine treatments on several of the inoculated produce items evaluated, complete elimination was not achieved. Nguyen-the and Carlin (1994) reported that the elimination of organisms associated with the surface of fruits and vegetables by the application of chlorine solutions is limited and unpredictable due to factors such as the surface structure of the produce and the presence of organic matter. These limitations were exemplified by the responses of *E. coli O157:H7* and *S.* Montevideo used in this study, with populations exhibiting a greater degree of sensitivity to chlorine treatments when inoculated on cantaloupe in comparison to lettuce An incremental degradation in the challenge organism populations was not observed with the increasing chlorine concentrations (in most cases) in this project While perplexing these results further highlight the variability both in response to disinfection treatments by the pathogens evaluated and the inherent variables associated with produce These variables include surface conditions presence of organic matter and the populations of the normal flora of the produce, all of which may influence the efficacy of treatments

Chlorine dioxide treatments The scientific information currently available on the effectiveness of chlorine dioxide is scant when compared to the quantity and quality of information available about the use of chlorine as a disinfectant for produce Treatment of inoculated strawberries with 100ppm chlorine dioxide proved to be the most effective solution examined to reduce counts of S Montevideo with reductions of 2 20 $\log_{10}$ CFU/ml obtained Treatment of inoculated tomato stem scars with 100ppm chlorine dioxide was not particularly effective on reducing populations of *E coli O157 H7*, producing reductions similar to tap water treatments Reductions in *S.* Montevideo populations on tomatoes after treatment with the 100ppm chlorine dioxide solutions at 43°C were not significantly different from any of the chlorine treatments evaluated, with only a 0 77 $\log_{10}$ reduction demonstrated

One $\log_{10}$ or greater reductions in the challenge bacteria was observed with the commercial chlorine dioxide product for *S* Montevideo populations on romaine lettuce. The *E. coli O157:H7* on the lettuce proved more resistant to this treatment, with only a 0.66 $\log_{10}$ reduction obtained. Treatment of inoculated cantaloupe with 100ppm chlorine dioxide, in comparison, produced reductions in excess of 2.5 $\log_{10}$ units in both *E. coli O157:H7* and *S.* Montevideo populations.

To date there are no scientific studies published that explore the effects of stabilized chlorine dioxide products on viruses associated with produce surfaces. Treatment of inoculated strawberries with 100ppm chlorine dioxide resulted in almost a three $\log_{10}$ PFU/ml reduction (2.95) for the MS2 populations, while a 1.55 and 1.68 $\log_{10}$ PFU/ml reduction were achieved for PRD-1 and Phi-X 174, respectively, with the chlorine dioxide treatments. Tomato stem scars treated with 100ppm chlorine dioxide demonstrated that the compound was not particularly effective on reducing populations of PRD-1 and MS2, producing reductions similar to tap water treatments. Phi X reductions in response to chlorine dioxide treatments were comparable to those observed with the chlorine treatment solutions on tomato stem scars.

The polio 1 viruses inoculated onto lettuce demonstrated no significant difference in response to treatments with 50ppm free chlorine and 100ppm of the Carnebon 200® product. The viruses, in general, were not reduced efficiently with the chlorine dioxide treatments, with PRD-1, Phi X 174, and polio virus each reduced less than 1 $\log_{10}$ PFU/ml. MS2 populations, however, were reduced 1.22 $\log_{10}$ in response to treatment with the chlorine dioxide product at 100ppm and appeared to be the most sensitive of the viruses examined to this treatment when associated with lettuce.

Treatment of inoculated cantaloupe with the Carnebon 200® product at 100ppm produced greater reductions in the challenge viruses than in the other produce types. Cantaloupe treated with the 100ppm chlorine dioxide solution produced reductions greater than 2.5 $\log_{10}$ units in PRD-1 and Phi-X 174 populations. The phage MS2 proved to be the most resistant to the chlorine dioxide treatment, with only a 1.8 $\log_{10}$ reduction obtained, which was not significantly different from the reduction achieved with a tap water treatment.

Chlorine dioxide has been used to disinfect *Listeria monocytogenes* inoculated onto lettuce by Zheng and Farber (1996), who achieved a 0.8-$\log_{10}$ reduction when 5.0ppm was applied at 22°C. Chlorine dioxide has also been evaluated for controlling microorganisms in water used in hydrocoolers used for chilling cucumbers (Reina et al., 1995). Beuchat (2000) indicates that as with other disinfectants, microorganisms frequently vary in their response to treatment with chlorine dioxide, and environmental factors as well as the method of application can influence efficacy, but that further research on this compound is warranted. The extremes observed in response to treatment with this product are clear when the challenge organisms are examined by produce item. When looking at the ranges of reductions across the 4 produce types, MS2 populations were reduced in amounts ranging from 1.22 – 2.95, whereas PRD-1 population reductions varied from 0.35 – 2.68 and Phi X log$_{10}$ reductions ranged from 0.30 to 2.93. The challenge bacteria demonstrated similar variations in response to the chlorine dioxide treatments. This study supports other researcher's reports that challenge organisms will vary in response to treatment with chlorine dioxide and that produce type and physical structure may influence the efficacy. However, the statistically significant reductions that were demonstrated on challenge organism populations attached to the seeded produce support the need for further research with chlorine dioxide compounds.

Persistence of challenge organisms on strawberries. Persistence of challenge organisms was examined when held under typical storage conditions encountered in food service and home settings. Organisms were applied to strawberries using both deionized water and a filtered sewage carrier to examine the effect of the introduction of nutrients and organics that are present in wastewater. The use of wastewater and the application of inadequately composted manure to soils used to produce fruits and vegetables is a concern about fruits and vegetables produced both domestically as well as products imported from abroad. The ability of both viruses and bacteria to persist in contaminated soils, as well as on adulterated products grown on contaminated soils or irrigated with untreated wastewater has been well documented (Sadovski et al., 1978; Tierney et al., 1977). The results of this study indicate that the challenge organisms survived better when inoculated via the deionized water carrier when compared to the wastewater carrier. The presence of nutrients and organic matter associated with wastewater are frequently reported to attribute to the increased persistence of challenge organisms (Sadovski et al., 1978 Tierney et al 1977) The benefits attributed to wastewater and associated nutrients provided to challenge organisms in previous studies was not realized in this case One factor in the decrease in of *E coli* O157 H7 and S Montevideo on the surfaces of strawberries may be that these introduced organisms were out-competed for available resources by the natural flora of the fruit Bacteria persist and actually thrive on plants as an integral part of natural microbial ecosystems Normal flora on produce items have been reported to be eluted in quantities ranging from 10^{6}-10^{10} (Upper et al., 1989) Preliminary experiments in this study included total aerobic plate counts which were performed using a nonselective media The strawberries examined were found to have counts in excess of 10^{8} CFU/ml (data not shown) The populations of organisms composing the plant-associated ecosystems have been described as dynamic, with the organisms being subjected to a variety of harsh environmental conditions (Upper et al 1989) This normal flora may be conditioned to not only survive harsh conditions, but also make efficient use of any available nutrients that may be present and multiply rapidly

Persistence of challenge organisms on tomatoes Human pathogenic bacteria or viruses that are not an established resident on plant surfaces are considered transient organisms (Upper et al , 1989) These transient organisms are subjected to the same harsh conditions resident flora must endure as a part

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of plant-associated ecosystems. This study examined the persistence of challenge organisms on tomatoes as influenced by inoculum carrier, location and storage temperature.

The influence of inoculum (deionized water and filtered sewage) was evaluated on tomatoes stored at 4 and 25°C. *E. coli O157:H7* applied to tomato stem scars in a water carrier declined 2.5 logs after 13 days and declined 3 logs after 16 days post-inoculation in a sewage suspension. *S.* Montevideo populations had dropped below detectable limits by day 13 with the water carrier but viable colonies were obtained on day 16 with the sewage inoculum, perhaps as influenced by the nutrients available in the sewage carrier. Wei et al. (1995) reported the survival of *S.* Montevideo on tomato stem scars for at least 7 days when stored at 25°C and reported that the *S.* Montevideo in a TSB carrier promoted better survival and protected against the bactericidal properties of aqueous chlorine treatments. While the bacteria included in this study had been washed in saline to remove any remaining TSB, the sewage carrier may have provided nutrients that may have contributed to the survival of the challenge organisms. Viruses will not grow in or on produce, but may persist long enough to be consumed with the produce. Viral agents deposited on the surfaces of plants are subjected to the same environmental conditions as the transient bacteria. However, because the viruses do not compete with the established flora for nutrients, and are essentially inert, they may actually be more stable than introduced human bacterial pathogens on these surfaces. The challenge viruses were deposited on the stem scars in a sewage carrier or suspended in deionized water and stored at 4 and 25°C. The sewage carrier applied PRD-1 and MS2 phages each declined in population 3 $\log_{10}$ units after 16 days of storage. The polio virus only declined 2 $\log_{10}$ units under the same conditions. When these agents were applied in a water medium, the MS2 phage populations declined in a near linear fashion, and were recovered at day 9, but were not recoverable 13 days post-inoculation. PRD-1 and polio virus on the other hand survived well, and only declined 1.25 and 1 $\log_{10}$ respectively after 13 days.

The challenge bacteria behaved more erratically when stored at 25°C than at 4°C. Both bacteria declined at least 2 $\log_{10}$ one day after inoculation via the water carrier, but demonstrated an increase in population on days 2 and 3. The populations dropped below detectable limits on days 6 and 7, but were recovered in measurable amounts on day 13. The challenge bacteria applied in the sewage medium both dropped 5 $\log_{10}$ one day after inoculation, but recovered at least 2 $\log_{10}$ by day 8. The *S.* Montevideo populations declined below detectable limits on day 11 and remained undetectable at day 16. The *E. coli O157:H7* populations were measurable at day 11, but were not detected after day 11. The application via sewage carrier produced viable *S.* Montevideo on tomato stem scars, which was similar to results reported by Wei et al. (1995) using a TSB suspended culture. The findings of this study indicate that viruses in general declined rapidly when applied in a sewage carrier, but both MS2 and PRD1 were detected at day 16. Polio virus was detected at day 11 but declined below levels of sensitivity by day 16 The viral agents inoculated via water also declined rapidly and both phages were below detectable limits by day 13 Polio virus persisted through day 7, which was the last day tested for that virus due to handling errors with the samples for the remaining time points

Separate experiments were performed to evaluate the persistence of bacteria on wax and un-waxed tomatoes The "wax" applied to the surfaces of tomatoes is typically a vegetable oil-based wax which serves to reduce chaffing to the tomato skins during shipment and subsequent handling (Rushing et al 1996) *E coli O157 H7* and *S* Montevideo were applied to waxed and un-waxed tomato stem scars with a water carrier and stored at 10°C Challenge bacteria persisted well on both tomato types and no significant reductions were observed in populations after 21 days of storage Zhuang et al (1995) reported observing no significant decreases in populations of *S* Montevideo on stem scars stored at 10°C for 18 days Intact areas of tomato surfaces were also inoculated as described above The bacteria populations declined below detectable limits on days 7 and 14 but both *E coli O157 H7* and *S* Montevideo were recovered from waxed tomatoes 21 days post inoculation This recovery at day 21 may be due to the clustering of the challenge orgnaisms resulting in better protection from the harsh storage environment, but only occurring on a small number of samples (see standard error bars Figure 17) The error bars from the waxed samples with positive recovery of bacteria did overlap the un-waxed samples

Wei et al. (1995) indicated that *S.* Montevideo populations persisted on damaged areas of inoculated tomatoes. Due to the difficulty associated with the great variation in the degree of damage naturally occurring on the surfaces of tomatoes, and the unknown degree to which these naturally occurring damaged areas will weep internal fruit juices, pin pricks of uniformed sizes were administered to the test fruit. The sound, intact surfaces of both waxed and un-waxed tomatoes were punctured with a sterile needle, inoculated, and stored at 10°C. Both challenge organisms persisted through the 21 day study. *S.* Montevideo populations on both tomato types increased over the 21 days. The puncture wound inflicted by a 25-gauge needle was scarcely observable to the naked eye, with no visible weeping of internal fruit juices observed on the surface prior to inoculation. However, this wound apparently was sufficient to influence the challenge organisms, enabling them to persist days longer on intact tomato surfaces.

Persistence of challenge organisms on romaine lettuce. The persistence of challenge organisms on romaine lettuce when held under typical storage conditions encountered in food service and home settings may be especially troubling to consumers and food safety stakeholders as well. While the inoculum levels of pathogen used in this study may not be encountered on commercially processed lettuce, the presence of viable cells at any level can be a concern due to their low infective doses (CAST, 1994). The viruses examined survived well at 4°C with the moist, cool environment providing a stable environment for these challenge organisms, which have been shown capable of surviving much harsher and more antagonistic environments (Sadovski et al., 1978, Tierney et al., 1977). The challenge bacterial pathogens also persisted to the end of the storage trial dropping slightly less than 2 $\log_{10}$ units after 10 days at 4°C.

Persistence of challenge organisms on cantaloupes. The persistence of challenge organisms were examined in this study on artificially contaminated cantaloupes when held under typical storage conditions that would be encountered in food service and home settings. Melons stored at 4°C had recoverable numbers of all challenge organisms 10 days following inoculation. Ukuku and Sapers (2000) reported the recovery of *S. stanley* after 5 days stored at >5°C, even after treatment of the inoculated melons with chlorine or hydrogen peroxide washes prior to storage. *E. coli O157:H7* and all three of the viruses were recovered after 30 days. The condition of the melons after 30 days of storage had deteriorated to the point that they most likely would not be purchased or consumed. The recovery of the challenge organisms after 10 days of storage may be of a more realistic concern to consumers, but the implications of the challenge organisms persisting for such a prolonged period cannot be ignored.

Conclusions. While numerous international studies have been published documenting the recovery of human pathogens from the various produce types (Beuchat, 2000), the information pertaining to the prevalence of specific pathogens and viruses on products destined for fresh market, and perhaps more importantly the concentrations of these pathogens is needed. This lack of critical information pertaining to the concentrations of pathogens that may be encountered on contaminated produce makes the design of realistic challenge studies difficult. The inoculum levels of the bacterial pathogens and model viruses used in this study may not be encountered on produce destined for the fresh market. However, pathogenic bacteria and viruses are often excreted in extremely high concentrations in the fecal matter of infected individuals, and therefore infected food handlers or farm workers may potentially contaminate produce with large numbers of pathogens (Oliver, 1997; CAST, 1994). Regardless of initial concentration, presence of viable pathogens in any amount can be troubling due to the low infective doses of some of these organisms such as 10^2 viable CFU/gm of *E. coli O157:H7* (CAST, 1994).

Both the viruses and bacteria persisted when applied via a sewage carrier to surfaces of strawberries and tomatoes. This carrier was included in this study because sewage may be come into contact to tomato and strawberry plants when improperly treated wastewater is used for irrigation purposes, or is applied to soils as a fertilizer. The challenge organisms also persisted on the produce items when inoculated in a water vehicle. This water inoculation may be representative of conditions in a commercial dump tank where sanitizer levels are not maintained in sufficient amounts to inactivate pathogens accumulating in the wash water. These conditions may also occur during batch washing of products at the retail or home level. Through this water suspension, pathogens may gain access to the produce items.

This study clearly demonstrated that the challenge organisms persisted on tomato stem scars and strawberries for extended periods of time and were resistant to the disinfection treatments applied. The ability for strawberries to promote the spread of viral illness has been well documented. The ability of pathogens inoculated on stem scars of tomatoes to be transferred to the interior of the fruit during slicing has also been demonstrated (Lin and Wei, 1997) as well as their implication in foodborne illness outbreaks. These points, when combined with the results presented within this study further demonstrate the potential for tomatoes and strawberries to serve as vehicles of foodborne illness.

It was also demonstrated in this study that that pathogenic bacteria and reference viruses can attach to various regions of precut romaine lettuce, and can be eluted in high numbers utilizing the rotary shaking method described. Cantaloupes were also found to facilitate the attachment and persistence of the challenge organisms. The challenge organisms were resistant to the treatments applied, and persisted well at storage temperatures on both lettuce and cantaloupe.

While the challenge organisms applied to cantaloupe were somewhat resistant to the treatments used, the treatments were more effective in producing greater reductions in the challenge bacteria and viruses than on the other produce types. The importance of protecting the melons from contamination during production, distribution, and at point of use cannot be stressed strongly enough. However, the careful removal of stem and blossom scars prior to slicing the melons may greatly decrease the potential for the introduction of pathogens to the edible tissues and further exploration of this method as a preventative treatment should be conducted.

In conclusion, this study illustrates that the pathogenic bacteria and model viruses examined attached to strawberries, tomatoes, lettuce and cantaloupe, persisted over a range of storage temperatures, and resisted common disinfection techniques. These findings emphasize the need for further scientific research into effective disinfection methods to address potentially contaminated fruits and vegetables. Additionally, findings demonstrate that further development of preventative measures for use in the fields as well as by processors may be necessary to prevent contamination of produce. The produce industry currently takes many precautions to prevent the contamination of the fruit including the use of good agricultural practices (GAPs) in the fields, and adherence to good manufacturing practices (GMPs) during processing. Implementation of the precautions outlined in the U.S. Food and Drug administration's "Guide to minimize microbial food safety hazards for fresh fruits and vegetables" may also play a key role in addressing produce safety both for domestic and international producers. However, consumers must do their part as well to prevent contamination at home by avoiding cross contamination with raw meat products and utilizing sanitary food handling practices

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APPENDIX

FLOW CHARTS OF EXPERIMENTAL PROCEDURES

FLOW CHARTS OF EXPERIMENTAL PROCEDURES

Strawberry Disinfection Procedures

Inoculation Preparation
18-hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2-µm porosity filter
⇓
Challenge organisms added to 2L of water carrier to produce 10^7 CFU or PFU/ml. Water temperature ≥ 15°C above temperature of fruit
⇓
25-30gm sound strawberries selected, stem/leaves removed
⇓
Immersed in inoculm 2 minutes with stirring, removed and dried 3 hours in biological safety cabinet
⇓
Treatment Solution Preparation
Solutions of 50, 100, 200, and 300ppm free chlorine were prepared using household bleach (5.25% sodium hypochlorite) with concentrations monitored using a HACH Free Chlorine Kit and solutions adjusted to a final pH of 7.3 by the addition of 1.0 molar hydrochloric acid. Carnebon 200® was prepared per label instructions. All treatment solutions were prepared in 2L volumes, and applied at 43°C
⇓
Treatment Application
Treatments were applied by immersion of 5 inoculated samples in the solutions for 2 minutes with constant manual stirring, followed by 45 second immersion in deionized water containing 0.05% (w/v) sodium thiosulfate
⇓
Sample Elution
Individual strawberries placed in 250ml sample bag containing 30mls PBS-Tween and placed on rotary shaker (60rpm) in 36°C incubator for 25 minutes
⇓
Samples diluted as necessary in 0.1% TSB
⇓
Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV
⇓
Plates counted after 24 hours at 36°C

Strawberry Persistence Procedure

Inoculation Preparation
18 hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre filtered with 0.2-µm porosity filter
⇓
Challenge organisms added to 2L of water or sewage carrier to produce 10^7 CFU or PFU/ml Carrier temperature ≥ 15°C above temperature of fruit

⇓ Deionized Water

⇓ Raw Sewage - filtered with 0.2-µm porosity filter

⇓
25-30gm sound strawberries selected, stem/leaves removed
⇓
Immersed in inoculm 2 minutes with stirring
⇓
Berries dried 3 hours in biological safety cabinet
⇓
Samples stored at 4°C
⇓
Sample Elution
Individual strawberries placed in 250ml sample bag containing 30mls PBS-Tween and placed on rotary shaker (80rpm) in 36°C incubator for 25 minutes
⇓
Samples diluted as necessary in 0.1% TSB
⇓
Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV
⇓
Plates counted after 24 hours at 36°C

Tomato Disinfection Procedure

Inoculation Preparation
18 hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2-µm porosity filter
⇓
Challenge organisms added to 2L of water or sewage carrier to produce 10^7 CFU or PFU/ml. Carrier temperature ≥ 15°C above temperature of fruit
⇓

Mature representative tomatoes (<24 hours after harvest) selected
⇓
25-µl inoculum deposited on respective site (stem scar, intact surface, pin-prick)
⇓
Fruit dried 3 hours in biological safety cabinet
⇓
Treatment Solution Preparation
Solutions of 50, 100, 200, and 300ppm free chlorine were prepared using household bleach (5.25% sodium hypochlorite) with concentrations monitored using a HACH Free Chlorine Kit and solutions adjusted to a final pH of 7.3 by the addition of 1.0 molar hydrochloric acid. Carnebon 200® was prepared per label instructions. All treatment solutions were prepared in 2L volumes, and applied at 43°C
⇓
Treatment Application
Treatments were applied by immersion of 5 inoculated samples in the solutions for 2 minutes with constant manual stirring, followed by 45 second immersion in deionized water containing 0.05% (w/v) sodium thiosulfate
⇓
Sample Elution
Inoculated sample regions excised with an ethanol-sanitized scalpel and placed in sterile centrifuge tubes containing 5.0ml PBS-Tween
⇓
Samples diluted as necessary in 0.1% TSB
⇓
Bacteria plated on TSA-rif w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV
⇓
Plates counted after 24 hours at 36°C

Tomato Persistence Procedure

Inoculation Preparation
18 hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2-µm porosity filter
⇩
Challenge organisms added to 2L of water or sewage carrier to produce 10^7 CFU or PFU/ml. Carrier temperature $\geq$ 15°C above temperature of fruit

⇩ ⇩
Deionized Water Raw Sewage - filtered with 0.2-µm porosity filter

⇩
Non-ripe representative tomatoes (pinks to green) selected
⇩
25-µl inoculum deposited on respective site (stem scar, intact surface, pin-prick)
⇩
Fruit dried 3 hours in biological safety cabinet
⇩
Samples stored at 4, 10, or 25°C
⇩
Sample Elution
Inoculated sample regions excised with an ethanol sanitized scalpel and placed in sterile centrifuge tubes containing 5.0ml PBS-Tween
⇩
Samples diluted as necessary in 0.1% TSB
⇩
Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV
⇩
Plates counted after 24 hours at 36°C

Lettuce Disinfection Procedure

18-hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2-μm porosity filter

⇩

Challenge organisms added to 5ml of deionized water carrier to produce 10^7 CFU or PFU/ml. Carrier temperature ≥ 15°C above temperature of lettuce

⇩

Head of romaine lettuce cut into approximately 6 x 8cm portions and mixed well placing 100g of lettuce placed into sterile stomacher bags (380 x 508cm)

⇩

3.0ml of the inoculum cocktail sprinkled across the lettuce, the bag folded and shaken to distribute the inoculum and samples stored at 4°C

⇩

Solutions of 50, 100, 200, and 300ppm free chlorine were prepared using household bleach (5.25% sodium hypochlorite) with concentrations monitored using a HACH Free Chlorine Kit and solutions adjusted to a final pH of 7.3 by the addition of 1.0 molar hydrochloric acid. Camebon 200® was prepared per label instructions. All treatment solutions formulated in 2L volumes, and were applied at 43°C

⇩

Treatments were applied by placing 100gms of the inoculated sample into 2L of treatment solutionsfor 2 minutes with constant manual stirring, followed by 45 second immersion in deionized water containing 0.05% (w/v) sodium thiosulfate

⇩

Inoculated sample (100gm) divided into 20gm samples placed were placed into sterile 250ml sample bags, with 60mls of PBS-Tween added to each sample bag

⇩

Samples placed on rotary shaker (60rpm) in 36°C incubator for 25 minutes

⇩

Samples diluted as necessary in 0.1% TSB

⇩

Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV

⇩

Plates counted after 24 hours at 36°C

Lettuce Persistence Procedure

Inoculation Preparation
18 hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2 μm porosity filter

⇓

Challenge organisms added to 5ml of deionized water carrier to produce 10^7 CFU or PFU/ml Carrier temperature ≥ 15ºC above temperature of lettuce

⇓

Head of romaine lettuce cut into approximately 6 x 8cm portions and mixed well, discarding inedible base/core

⇓

100g of processed lettuce placed into sterile stomacher bags (380 x 508cm)

⇓

3.0ml of the inoculum cocktail sprinkled across the lettuce the bag folded and shaken to distribute the inoculum

⇓

Samples stored at 4°C

⇓

Sample Elution
Inoculated sample (100gm) divided into 20gm samples placed were placed into sterile 250ml sample bags with 80mls of PBS-Tween added to each sample bag

⇓

Samples placed on rotary shaker (60rpm) in 36ºC incubator for 25 minutes

⇓

Samples diluted as necessary in 0.1% TSB

⇓

Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV

⇓

Plates counted after 24 hours at 35ºC

Cantaloupe Disinfection Procedures

Inoculation Preparation

18-hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2 µm porosity filter

⇓

Challenge organisms added to 5ml of deionized water carrier to produce 10^7 CFU or PFU/ml. Carrier temperature ≥ 15°C above temperature of fruit

⇓

1.5cm^2 areas were marked on the surfaces of the melons with a permanent marker. 50-µl inoculum deposited on marked surface and dried 18 hours in biological safety cabinet

⇓

Treatment Solution Preparation

Solutions of 50, 100, 200, and 300ppm free chlorine were prepared using household bleach (5.25% sodium hypochlorite) with concentrations monitored using a HACH Free Chlorine Kit and solutions adjusted to a final pH of 7.3 by the addition of 1.0 molar hydrochloric acid. Carnebon 200® was prepared per label instructions. All treatment solutions were prepared in 2L volumes and applied at 43°C

⇓

Treatment Application

Treatments were applied by immersion of individual inoculated melons in the solutions for 2 minutes with constant manual stirring, followed by 45-second immersion in deionized water containing 0.05% (w/v) sodium thiosulfate

⇓

Sample Elution

Inoculated sample regions excised with an ethanol-sanitized scalpel and placed in sterile centrifuge tubes containing 7.0ml PBS-Tween and placed on rotary shaker (60rpm) in 36°C incubator for 25 minutes

⇓

Samples diluted as necessary in 0.1% TSB

⇓

Bacteria plated on TSA-rif w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV

⇓

Plates counted after 24 hours at 36°C

Cantaloupe Persistence Procedure

Inoculation Preparation
18 hour cultures of challenge bacteria washed in 0.85% saline
Viral stocks pre-filtered with 0.2-µm porosity filter
⇓
Challenge organisms added to 5ml of deionized water carrier to produce 10^7 CFU or PFU/ml. Carrier temperature $\geq$ 15°C above temperature of fruit.
⇓
1.5cm^2 areas were marked on the surfaces of the melons with a permanent marker
⇓
50-µl inoculum deposited on marked surface
⇓
Fruit dried 18 hours in biological safety cabinet
⇓
Samples stored at 4 or 25°C
⇓
Sample Elution
Inoculated sample regions excised with an ethanol sanitized scalpel and placed in sterile centrifuge tubes containing 7.0ml PBS-Tween and placed on rotary shaker (60rpm) in 36°C incubator for 25 minutes
⇓
Samples diluted as necessary in 0.1% TSB
⇓
Bacteria plated on TSA-nf w/supplements
Viruses enumerated using soft agar overlay method on PCA-CV
⇓
Plates counted after 24 hours at 36°C

BIOGRAPHICAL SKETCH

Michael Bradley received his Bachelor of Science degree in food technology from Texas Tech University in December 1990. He then worked for the Texas Department of Health as a Food and Drug Investigator until coming to the University of Florida in the spring of 1997. He is married to Jennifer Bradley, with one son, Eli.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality as a dissertation for the degree of Doctor of Philosophy

Douglas L Archer, Chair
Professor of Food Science and Human Nutrition

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

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